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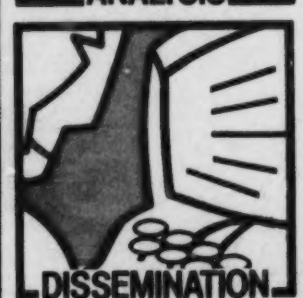

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CURRENT CANCER RESEARCH

on

TUMOR IMMUNOGENETICS AND RELATED TRANSPLANTATION BIOLOGY

AUGUST 1981

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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CURRENT CANCER RESEARCH

I. IMMUNOGENETIC STUDIES OF SPECIFIC TYPES OF CANCERS

1. IMMUNOGENETIC STUDY OF BREAST CANCER AND LEUKEMIA

Boot, L. M., Nederlands Kankerinstituut, *106-108 Sarphatistraat, Amsterdam, Netherlands*

The Contractor will conduct immunogenetic studies on the hereditary transmission of the mammary tumor virus in the GR strain in which the genetic influence associated with breast cancer is similar to that observed in humans.

2. IMMUNOGENETIC RESISTANCE TO LYMPHOMA-LEUKEMIA

Trentin, J. J., Baylor College of Medicine, School of Medicine, Dept. of Surgery, *1200 Moursund Ave., Houston, Texas,* 77025, United States of America

OBJECTIVE: To investigate and better understand the mechanisms responsible for genetic resistance to bone marrow transplantation; genetic resistance to lymphoma transplantation; genetic resistance to virally induced or spontaneous lymphoma-leukemia; and spleen natural killer cell lysis of lymphoma cells in vitro. The possible identity of these mechanisms and their applicability to the prevention of lymphoma-leukemia will be investigated.

BIBLIOGRAPHIC REFERENCES: Datta, Surjit K., Michael T. Gallagher and John J. Trentin. Natural cell-mediated cytotoxicity in hamsters. Int. J. Cancer 23: 728-734, 1979. Datta, S.K., M. T. Gallagher, J.J. Trentin, R. Kiessling, and H. Wigzell. Apparent identity of mechanisms of genetic resistance to marrow transplantation and natural killer cell activity. Biomed. Express 31:62-66, 1979.

3. GENETIC CONTROL OF RESISTANCE TO HERPESVIRUS-INDUCED LYMPHOMA

Longenecker, B. M., Medical Research Council of Canada, Group on Immunoregulation, *Edmonton, Alberta, Canada*

OBJECTIVE: To determine the mechanism of genetic resistance to Marek's disease.

APPROACH: (a) Irradiated and non-irradiated thymus glands from susceptible (line-7) chicks are transplanted to resistant (line 6) thymectomized chicks and exposed to MDV to determine whether the presence of susceptible target T-lymphocytes will make a resistant chick more susceptible. (b) Metastatic variants of lymphoma cell lines derived from MD tumors are selected. Mice are immunized with these variant cell lines and hybridomas which distinguish the variants are produced. The monoclonal antibodies secreted by these hybridomas which detect important cell surface markers associated with metastasis and viral receptors are the most useful.

PROGRESS: Several hybridomas which distinguish the metastatic variants have been produced and are being screened. The presence of a susceptible (line-7) thymus microenvironment in genetically resistant animals makes the resistant animal more susceptible.

4. IMMUNOGENETIC STUDY OF A HERPESVIRUS INDUCED LYMPHOMA

Schierman, L. W., Kleven, S. H., Fletcher, O. J., University of Georgia, School of Veterinary Medicine, Dept. of Avian Medicine, *Athens, Georgia,* 30601, United States of America

The primary objective of this proposal is to analyze the factors which allow for progressive growth of malignant Marek's disease virus (MDV)-induced tumors in chickens which have a demonstrable immune response to their tumors. Thus, the major goals will be to determine: a) what cellular and humoral immune responses are elicited in birds with primary or non-transplantable Marek's disease (MD) lymphomas as well as birds possessing a syngeneic transplantable MD lymphoma; b) what mechanisms may exist by which MD lymphomas escape from host immune responses; and c) if procedures might be developed which would repress mechanisms responsible for escape of MD lymphomas from host immunity. Information obtained from previous studies showing limited transplantability of most MDV-induced primary tumors passaged into syngeneic hosts will be utilized to further explore the host response to tumors. A highly malignant transplantable MD lymphoma, developed in one of our inbred lines, will also be used to study immunopathological properties associated with tumor invasion and metastasis. The availability of inbred lines of chickens in which we can do histocompatible cell transfers, the use of a syngeneic transplantable lymphoma and the development of vaccines highly effective against MD offer unique advantages for studying tumor and host factors affecting growth of herpesvirus induced lymphomas. Particular emphasis will be placed on studying ways to counteract subversion of host reactivity to progressively growing lymphomas.

BIBLIOGRAPHIC REFERENCES: Schierman, L.W. and R.A. McBride. Effects of vaccination for Marek's disease on growth of a Marek's-virus-induced transplantable lymphoma in syngeneic and allogeneic chickens. Avian Diseases 23: 47-56, 1979. Schierman, L.W. and O.J. Fletcher, Genetic control of Marek's disease virus-induced transient paralysis: association with the major histocompatibility complex Proc. E.E.C. Symposium on Resistance and Immunity to Marek's Disease, West Berlin, Oct. 31 - Nov. 2, 1978, (In press).

5. GENETIC BASIS OF SJL/J MURINE RETICULUM CELL SARCOMA

Bubbers, J. E., University of California, Los Angeles Campus, School of Medicine, Dept. of Radiation Oncology, *2025 Zonal Ave., Los Angeles, California,* 90024, United States of America

Mice of the SJL/J strain demonstrate a high incidence (90 percent at 13 months) of spontaneous reticulum cell sarcoma (RCS). It is the intent of this project to initiate studies which will establish the underlying genetic basis for this malignancy and, in so doing, determine the nature and the number of the genes involved. This will be accomplished by the application of Mendelian genetic principles to the rates of disease incidence in SJL/J and hybrid mice. Progress to date in the understanding of the disease process has been hindered by the multicellularity of the tumor and the lack of obvious association with murine leukemia virus expression. By breeding SJL/J mice with normal strains to produce F1 mice, the dominant or recessive nature of disease expression will be determined. Subsequent matings of F1 mice to parental strains will produce backcross mice in which the incidence of RCS will be dependent upon the number of independently segregating disease-associated genes. The number of these genes will be indicated by relative rates of RCS in SJL/J and backcross mice. Two loci in particular (Fv-1 and H-2) will be examined for their influence on RCS because of previously demonstrated effects of these genes on other murine lymphoreticular tumors. Mice of SJL/J backcross populations in which the normal parent is not H-2s will be typed by hemagglutination and followed for cosegregation of resistance to RCS with H-2 heterozygosity. Additionally, mice of SJL/J backcross populations in which the normal parent differs from SJL/J both at Fv-1 and the tightly linked gene Gpd-1, will be Gpd-1 typed by starch gel electrophoresis and followed for cosegregation of resistance to RCS with Gp-1 (and hence Fv-1) heterozygosity.

6. GENETIC CONTROL OF IMMUNE RESPONSE TO METHYLCHOLANTHRENE-INDUCED MURINE FIBROSARCOMAS

Parmiani, G., Ballinari, D., Sensi, M. L., Istituto Nazionale per lo Studio e la Cura dei Tumori, Division of Experimental Oncology A, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: To study whether the immune response to individual tumor-associated antigens (TATA) of methylcholanthrene (MCA)-induced murine sarcomas is under control of genes of the major histocompatibility complex.

APPROACH: MCA-induced, TATA-bearing sarcomas of various inbred strains of mice will be used to evaluate the anti-TATA immune response of the strain of origin of the tumor in comparison with the immune response of different F1 semisyngeneic hybrids. The immune response will be evaluated both in vivo (growth, excision and challenge method) and in vitro (CML or 51Cr labelled tumor cells). Possible linkage between high or low immune responders and H-2 haplotype will be studied by backcross tests.

PROGRESS: Evidence for a polygenic control of the in vivo immunity to TATA of two BALB/c sarcomas has been obtained, involving both non-H-2 genes and products of the MHC.

7. IMMUNOGENETIC ANALYSIS OF ROUS SARCOMA DEVELOPMENT

McBride, R. A., Watanabe, D. H., Cutting, J. A., Chalmers, J. H., Baylor College of Medicine, School of Medicine, Dept. of Pathology, *1200 Moursund Ave., Houston, Texas,* 77025, United States of America

The objective is to define genes and mechanisms which control tumor progression or regression. By using closely related inbred lines of chickens which differ at MHC haplotypes and which differ in ability to regress ASV-induced tumors we can study the function of genes which control the expression of the resistance trait. By using ASV's which are genetically defined we hope to determine the nature of viral induced changes in tumor cells which determine whether a tumor progresses or regresses in a genetically defined host.

8. GENETIC CONTROL OF RESISTANCE TO MURINE PLASMACYTOMAS

Phillips Quagliata, J. M., New York University, School of Medicine, Dept. of Pathology, *100 Washington Sq. E., New York, New York,* 10003, United States of America

We are continuing our investigaton of hybrid resistance to the BALB/c plasmacytomas MPC-11, LPC-1 and MOPC-70A. We have discovered that resistance to MPC-11 depends on a single, dominant autosomal gene or gene complex which is not linked to the major histocompatibility H-2 complex of mice. We call this gene Rtp, for resistance to transplantable plasmacytoma. In the presence of Rtp positive, differential effects of the presence of different H-2 haplotypes in the hybrids may be seen. Susceptibility of hybrids to MPC-11 may involve either a negative allele of Rtp(positive), Rtp(negative), or expression in some hybrids of genes concerned with control of the GIX thymocyte differentiation antigen. We are currently studying resistance of typed backcross mice to determine whether Rtp is linked to either Ly-4 or Ea-4, possibilities suggested by our earlier work with F1 hybrids between BALB/c and the Bailey recombinant inbred strains. We are also completing similar studies on resistance to LPC-1 and are attempting to investigate the role of the H-2 complex in recognition of cross-reactive tumor associated transplantation antigens (TATA) in these tumors. We shall shortly initiate studies of the control of resistance to MOPC-70A which may not cross react with MPC-11 and LPC-1. We intend to investigate cellular mechanisms of susceptibility and resistance to the three tumors and to make a serological analysis of determinants expressed on the three tumors and in resistant and susceptible hybrids.

9. IMMUNOGENETIC CONTROL OF BRAIN TUMOR GROWTH IN RATS

Geyer, S. J., Gill, T. J., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Laboratory Service, *University Dr. C. Pittsburgh, Pennsylvania,* 15240, United States of America

PROJECT OBJECTIVES: To determine the immune response to a highly malignant brain tumor in both susceptible and resistant rats and to determine the genetic control of the immune response to this tumor.

PLAN: The tumor is maintained in tissue culture and injected into various rats of differing susceptibilites to the tumor. The tumor is injected into the peritoneal (abdominal) cavities of the rats because of the easy access of immune cells from this site. The reactive immune cells are characterized morphologically and functionally in a variety of in vitro and in vivo assays to determine if immunologic reactivity varies or correlates with tumor susceptibility or resistance. Various hybrid rats, produced from mating susceptible and resistant inbred parental strains are also studied for immune responsiveness to the tumor.

II. GENETICS OF THE IMMUNE RESPONSE

A. GENETIC CONTROL AND REGULATION OF THE IMMUNE RESPONSE

10. THE IMMUNOGENETICS OF MACROPHAGE SUPPRESSION IN MAN

Stux, S. V., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts,* 02115, United States of America

We propose to study in vitro a human suppressor monocyte/macrophage system. Our data which provide the background for this proposal show that suppressor monocyte/macrophages can be elicited by in vitro culturing of normal, peripheral blood mononuclear cells. These suppressor cells can be purified by Ficoll density gradient centrifugation. The cultured monocyte/macrophages are capable of suppressing allogeneic stimulation, generation of cytotoxic cells and mitogenic stimulation. We are able to extract a soluble factor from such cells which itself can suppress the mixed lymphocyte reaction. Using a panel of fully HLA-A, -B, -C and -DR typed normal donors, our objective is to: a) study the importance of HLA-DR (Ia) specificities at the level of suppressor monocyte/macrophages and at the level of suppressor soluble factors as each interacts with HLA-DR typed responder cells stimulated by alloantigen or by pokeweed mitogen (PWM). We shall measure alloantigen stimulation by 3H-thymidine incorporation and mitogenic stimulation by immunoglobulin production, using a radioimmunoassay. The suppressor values obtained in checkerboard-type cell combinations will be used to a) evaluate clustering of suppression values in relation to the number of HLA-DR specificities shared, i.e., two, one or none between each suppressor and responder cell pair; b) characterize the soluble factor from physical and immunochemical points of view; this will include: stability, molecular weight, solid phase immunoadsorptions by the use of Sepharose coupled xenoantisera directed against: human immunoglobulin, human beta 2 microglobulin, human p29,34 (Ia); c) establish the antigenic uniqueness of suppressor monocyte/macrophages from the point of view of cell surface differentiation antigens by preparing xenoantisera to these cells and by rendering them specific through successive adsorptions with Sepharose-pooled human serum, autologous red blood cells, autologous T cells, Daudi cells and eventual adsorption and elution from chemically fixed autologous suppressor monocyte/macrophage cells.

BIBLIOGRAPHIC REFERENCES: Stux, S., Hammond, P., Fitzpatric, C., Dubey, D. and Yunis, E., 1980. Use of Monocytes in HLA-A, B, C and DR Typing. Tissue Antigen 15:152. Stux, S., Dubey, D., and Yunis, E., 1980. Serological Relationship Between DR, DC and p29,34(Ia) Antigenic Determinants. Fed. Proc. 39:2221.

11. THE REGULATION OF THE ANTIBODY RESPONSE

Cinader, B., D'Agostaro, G. F., University of Toronto, Inst. of Immunology, *Medical Sciences Bldg., Toronto, Ontario,* M5S 1A8, Canada

Among the central problems in the analysis of any disease are (1) the process of causation and (2) the processes determining why the disease affects some individuals and not others and why it progresses more rapidly in some individuals than in others, i.e. the analysis of polymorphic factors which control incidence and progression of the disease. It has become apparent that some of the factors, relevant to the second of these prob-

lems, are to be found in the polymorphism of the immune response. The correlation between HLA haplotype and disease incidence has furnished indications of a link between MHC and disease incidence and/or progression. In addition, there are clearly other genetic levels of control, and particularly controls which are responsible for age dependent changes which occur at different individuals. Cancer is a disease with an incidence that is much greater in later than in early life. It is, therefore important to acquire insight into the polymorphism of these age-dependent changes, into methods by which they can be modified and into the extent to which they can be exacerbated by viruses and, particularly, by tumor viruses. In short, the question arises of how age-dependent changes in the immune response are regulated, how they affect incidence and progression of neoplastic disease and whether and how age-dependent changes in the immune system can be modified by tumor viruses. The work supported under this grant has been designed to this end; we examine polymorphic age-dependent changes in T suppressor cell circuits, the effect of drugs on the circuit, the effect of a tumor virus on the age-dependent changes. We are preparing the foundation for further studies on immune controls of tumor colony formation, in terms of age-dependent changes in the immune system; information, obtained by this type of analysis, may provide novel and more sophisticated approaches to immunotherapy.

12. GENETIC REGULATION OF THE IMMUNE RESPONSE

Longo, D. L., Paul, W., Schwartz, R., Dobson, P., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Medicine Branch, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

OBJECTIVES: Analysis of the expression of immune response genes in T cells, B cells, and antigen presenting cells. Study of the mechanism of action of the thymus on the development of T cell repertoire for antigen recognition and self-recognition. Experiments on the ontogeny of antigen presenting cells and their turnover in spleen, peritoneal cavity, and thymus.

Selected Highlights of Work Completed This Year: I. Gene Complementation: We have demonstrated that the genes which control the responsiveness to a given antigen need not be present in the genome of the responding T cell as long as it has matured in an environment in which the gene was present (Longo and Schwartz:J. Exp. Med., in press). We have also demonstrated that the mechanism by which a genetic nonresponder T cell 'learns' to respond to a given antigen is by developing a receptor for the antigen in conjunction with immune response gene products on the surface of the antigen presenting cell, i.e., the T cell sees both structures, antigen and Ir gene products (Longo and Schwartz: Proc. Natl. Acad. Sci., in press).

We have further shown that the alteration in the T cell receptor repertoire which occurs in the thymus may be caused by antigen presenting cells in the thymus. These thymic antigen presenting cells may determine what the T cell emerging from the thymus is capable of doing (Longo and Schwartz: Nature, in press).

Further studies have examined the celluar requirements for mounting an antibody response to T-independent antigens, of which the pneumococcal polysaccharide vaccine is an example (Kirkland et al.: J. Immunol.).

BIBLIOGRAPHIC REFERENCES: Longo, D.L. and Witherspoon, J.M.: Focal neurologic symptoms in hypercalcemia. Neurology 32: 200-201, 1980. Kirkland, T.M., Sieckmann, D.G., Longo, D.L., and Mosier, D.E.: Cellular requirements for antigen presentation in the induction of a thymus-independent antibody response in vitro. J. Immunol. 124: 1721-1726, 1980.

13. GENETIC CONTROL OF ALLOTYPE EXPRESSION OF HUMAN IG

Litwin, S. D., Cornell University, Medical Center, Cornell Medical College, Dept. of Medicine, *1300 York Ave., New York, New York,* 10021, United States of America

The synthesis of immunoglobulin in vitro will be studied in human peripheral blood lymphocytes after short-term culture with pokeweed mitogen. Secreted Ig will be measured by isotope radioincorporation and immunoprecipitation. The effect of prednisolone treatment on Ig synthesis and secretion will be analyzed at different levels of pokeweed mitogen. Cell separation experiments and irradiation will be used to examine the contribution of each cell type. The relationship between steroid sensitive cells in normal peripheral blood and steroid-sensitive suppressor cells in iummnodeficiency will be studied.

BIBLIOGRAPHIC REFERENCES: Characteristics of Suppressor Cell Activity Appearing in Cocultures of Two Individuals with Immunodeficiency with Thymoma, by Litwin, S.D. in Scand. J. Immunol. 11:15, 1980.

14. MECHANISM OF MAMMALIAN GENE EXPRESSION

Hozumi, N., Ontario Cancer Inst., Dept. of Biological Research, *500 Sherbourne St., Toronto, Ontario,* M4X 1K9, Canada

OBJECTIVE: The objective of this project is to study regulation of mammalian genes at the molecular level.

APPROACH: We obtain clones of immunoglobulin genes carrying promotor region V and C genes. These genes will be analyzed by using molecular and cell biological techniques.

PROGRESS: We have obtained DNA clones from a hybridoma cell line which has a specificity for hapten TNP. Gamma light chain clones were also obtained from variant types of M315. These variants include non-secretor and non-producer types. These clones are currently analyzed.

15. REGULATION OF ERYTHROID DIFFERENTIATION IN MURINE AND HUMAN LEUKEMIA AND THE GENETIC CONTROL OF HOST CELL RESPONSE TO LEUKEMOGENIC VIRUS

Axelrad, A. A., McLeod, D. L., Amato, D., Wu, A. M., Croizat, H., University of Toronto, Faculty of Medicine, Dept. of Anatomy, *92 College St., Toronto, Ontario,* M5S 1A8, Canada

We are investigating the mechanisms that regulate cell proliferation among early progenitors in the hemopoietic system, with the help of clonal cell culture methods. Our aim is to understand the operation of these mechanisms in normal individuals and how they become defective in the myeloproliferative disorders of mouse and man, so that improved strategies of management and prevention can be planned. We have found that a single gene Fv-2 (whose location is mapped on chromosome 9 in the mouse) controls both proliferation of an early progenitor cell (BFU-E) along the erythrocytic line of differentiation and susceptibility to Friend virus, which induces a primary polycythemia in mice. Very recently we have obtained evidence that BFU-E proliferation can be reversibly stopped through a macromolecular negative regulator from bone marrow whose production appears to be under the control of the same gene. We are presently trying to determine the nature and mechanism of action of this regulator and whether or not it also influences the growth of cells transformed by the polycythemia-inducing Friend virus.

16. GENETICS AND REGULATION OF NATURAL CELL-MEDIATED CYTOXICITY

Clark, E. A., University of Washington, Regional Primate Research Center, *c/o Health Sciences Ctr., Seattle, Washington,* 98195, United States of America

The objective of this research is to elucidate the genes, cells, and mechanisms involved in the regulation of natural cell-mediated cytotoxicity (NCMC). We will examine in the mouse both non-H-2 and H-2 genetic control of NCMC using congenic and recombinant inbred strains of mice. Gene complementation will be investigated in one system. An optimal protocol for in vivo and in vitro augmentation of NCMC will be established and applied in genetic analyses. The cellular requirements and characteristics of in vitro activation and suppression of NCMC will be investigated. Methods for selectively depleting or isolating natural killer (NK) cells will be developed so that NK cells can be added or removed from cultures in studies of the NK cell's role in T and B cell immune responses. Conventional antibodies and monoclonal-antibody-producing hybrid cell lines specific for NK-cell-associated antigens will be generated for this purpose. A nonhuman primate model for NCMC will be investigated. Family and in vivo augmentation studies will be performed. The characteristics of the primate NK cell and the specificity of NCMC against nonhuman lymphoblastoid cell lines will be investigated.

17. INDUCTION OF GENETICALLY TAILORED IMMUNE IMBALANCE

Leech, S. H., Louisiana State University, Medical Center, Dept. of Medicine, *New Orleans, Louisiana, United States of America*

The long-term goal of this project is to develop a new strategy for manipulating the immune system of a tumor-bearing host to produce an effective tumor-specific response. This strategy is based on the following three principles: 1. Hapten-help is the augmentation of the immune response to an antigen by challenging, with the antigen conjugated to a hapten, an individual previously sensitized to the hapten. For tumor immunotherapy, methods will be developed for the neoantigenization of tumor cells and for the conjugation of haptens to purified tumor specific antigen. Hapten priming for help is expected to expand a clone of hapten-specific T cells, not only capable of helping B cells produce cytotoxic antibody for complement-dependent lysis, but also of inducing other tumor-specific effector mechanisms, such as T cell-mediated lysis and antibody-dependent K cell-mediated lysis. All three effector mechanisms will be studied in this project by standard in vitro techniques. 2. Hapten priming is also expected to generate a population of hapten-specific suppressor cells. As these would suppress tumor-specific effector mechanisms, suppressor-inactivation will be accomplished by pretreatment with the hapten conjugated to a nonimmunogenic carrier, such as D-GL. 3. Ir genes determining the ability of inbred strains of mice to produce hapten help and suppressor inactivation will be investigated. The information thus obtained should be useful in the selection of a hapten suitable for the immunoprophylaxis and immunotherapy of tumor-bearing mice of each strain.

18. REGULATION OF THE IMMUNE RESPONSE IN ADULT ANIMALS TO ANTIGENS FIRST ENCOUNTERED IN THE NEONATE

Gorczynski, R. M., Ontario Cancer Inst., Division of Biological Research, *500 Sherbourne St., Toronto, Ontario*, M4X 1K9, Canada

OBJECTIVE: To investigate the effect of neonatal response to antigen on the response of adult animals on re-exposure to those antigens.

APPROACH: In order to understand these events we are currently investigating two different systems. In the first, we are studying the development of cytotoxic killer T cells into semiallogeneic cells in mice inoculated with those semiallogeneic cells at birth.

Tolerance in this case is to H-2 incompatible cells. In the second system we are analyzing the response of adult mice to antigens expressed on syngeneic embryo fibroblasts.

PROGRESS: Tolerance to MHC incompatible cells is maintained by suppressor cells. Two types of suppressor cells are detectable. The first acts to inhibit the induction of killer cells (from their immediate precursors) in culture. The second seems to be able to inhibit the production of cytotoxic cell precursors from (multipotential?) stem cells in vivo.

19. GENETIC CONTROL OF THE IMMUNE RESPONSE TO NATURAL ANTIGENS

Berzofsky, J. A., Richman, L. K., Berkower, I., Kohno, Y., Strober, W., Minna, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Metabolism Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The mechanisms of determinant-specific Ir gene control and of antigen recognition by immunocompetent cells (T and B lymphocytes) were explored at several levels in responses to myoglobin. Liver macrophages, pretreated with antigen and washed, were found to mediate Ir gene controlled presentation of selected antigenic determinants for T cell responses. Pretreatment of these cells with anti-Ia antibodies not only blocked this ability, but also induced suppression of responses by untreated cells. In vitro antibody responses to myoglobin were found to be under the same gene control, and to be T-cell and macrophage dependent. Myoglobin-specific receptors were isolated from T cells and shown to be distinct from immunoglobulin and able to bind myoglobin in the absence of Ia antigens. Monoclonal antibodies to myoglobin were prepared and their fine specificity for different determinants defined, in order to study their binding sites in comparison with the complementary sites on the antigen. Thus it is hoped to understand the complex genetically controlled regulatory interactions between T cells, B cells, and macrophages in the immune response and the biochemical nature of the several antigen-specific receptors involved.

BIBLIOGRAPHIC REFERENCES: Berzofsky, J.A., Richman, L.K., and Killion, D.J., Distinct H-2-linked Ir genes control both antibody and T cell responses to different determinants on the same antigen, myoglobin. Proc. Nat. Acad. Sci. USA 76: 4046-4050 (1979). Richman, L.K., Strober, W., and Berzofsky, J.A., Genetic control of the immune response to myoglobin. III. Determinant-specific, two Ir gene phenotype is regulated by the genotype of reconstituting Kupffer cells. J. Immunol. 124: 619-625 (1980).

20. GENETIC BASIS OF ANTIGENIC VARIATION

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The long-term objective of this project is to understand the genetic and epigenetic mechanisms by which the tumor cell regulates the expression of its tumor-associated antigens. Antigenic variants of murine lymphomas growing in tissue culture are being used to study the genetics of several model normal and tumor-associated cell surface antigens. Particular emphasis is being placed on understanding the Thy-1, T200 and TL glycoproteins. Mutant cell lines which do not express these glycoproteins on their cell surface are being isolated by cytotoxic immunoselection and characterized using a combination of somatic genetic and biochemical approaches. Hybrids between cell lines expressing Thy-1 and cell lines representing cell types which normally do not express Thy-1 are also under study. By this means the number and nature of the genes involved in the biosynthesis of a particular cell surface molecule are being defined.

BIBLIOGRAPHIC REFERENCES: A. Chapman, I. Trowbridge, R. Hyman, and S. Kornfeld (1979). Structure of the lipid-linked oligosaccharides that accumulate in Class E Thy-1-negative mutant lymphomas. Cell, 17, 509-515. I. Trowbridge, and R. Hyman (1979). Abnormal lipid-linked oligosaccharides in Class E Thy-1-negative mutant lymphomas. Cell, 17, 503-508.

21. IMMUNE RESPONSE TO RECOMBINANT ONCORNAVIRUS

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OBJECTIVES: Correlate precise genetic control of immune responsiveness of inbred mouse strains to different murine oncornavirus gp70 determinants. Determine the relative contributions of humoral immunity and classical cell-mediated immunity by cytotoxic T lymphocytes in affording protection against well-defined tumor antigens and tumor growth. Induce tumors in appropriate parental strains with cloned, characterized N- and B-tropic oncornaviruses. Test these tumors for growth in F1 hybrids constructed between the parent of origin and appropriate H-2 haplotype partner. Study the following parameters of immunological responsiveness determining host resistance to tumors: serum antibodies and tumor specific T lymphocytes. Identify distinct members of the gp70 family to which Ir gene controlled responses are directed.

22. GENETIC CONTROL OF THE IMMUNE RESPONSE TO STAPHYLOCOCCAL NUCLEASE

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Antibodies directed against idiotypic determinants on anti-Staphylococcal nuclease antibodies from different mouse strains have been produced in rats and in pigs. The idiotypes are detected by hemagglutination assays and by the inhibition of antibody-mediated inactivation of nuclease. By screening a variety of strains and offspring from appropriate matings between strains for the presence of idiotypes and other markers, it has been shown that idiotype expression is linked to the heavy chain allotype markers. A number of recombinants between different

idiotype markers have been found and may provide tools for fine-structure mapping of heavy chain variable region genes. Administration of anti-idiotypic antibodies to mice has been found to induce idiotype expression in the serum of these animals. This effect appears to involve T cells, since it is not observed in nude mice, and since evidence for idiotype expression on T helper cells in an in vitro anti-TNP nuclease response has also been obtained using spleens from such treated animals. Isoelectric focusing studies on antinuclease antibodies have indicated characteristic spectrotypes for different strains which appear to correlate with idiotype expression.

BIBLIOGRAPHIC REFERENCES: Schwartz, R. H., Berzofsky, J. A., Horton, C. L., Schechter, A. N., and Sachs, D. H.: Genetic control of the T-lymphocyte proliferative response to staphylococcal nuclease. Evidence for multiple MHC-linked Ir gene control. J. Immunol. 120: 1741-1749, 1978. Pisetsky, D. S., Riordan, S.E., and Sachs, D. H.: Genetic control of the immune response to staphylococcal nuclease. IX. Recombination between genes determining BALB/c antinuclease idiotypes and the heavy chain allotype locus. J. Immunol. 122: 842-846, 1979.

23. GENETIC REGULATION OF THE IMMUNE RESPONSE TO SYNTHETIC POLYPEPTIDES

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The research will employ synthetic polymers of amino acids (random and sequential) to study aspects of immunogenicity and genetic control of the immune response (Ir) in mice. The polymers have been T-cell dependent and 'histocompatibility-linked.' The strain distribution patterns in inbred mice will be studied. Immune responses will measure both antibody in the serum (antigen binding assays) and specific plaque-forming cells (B cells), and the in vitro stimulation of peritoneal exudate T lymphocytes (PETLES). Comparisons will be made of T cell and B cell specificities by reactions with related polymers. Using recombinant inbred strains of mice, the Ir genes controlling responses will be mapped to areas within the I subregion in the mouse H2 complex. Cellular aspects, notably the involvement of thymus-derived antigen-reactive cells (T cells) in unresponsiveness, i.e., suppressor T cells, T-cell antigen binding specificity, both at the cellular and subcellular levels, will be compared with the known humoral responses to the polymers. 125I-labelled polymers will be used in binding experiments with purified T cells obtained by nylon wool column techniques. The characterization of the nature of the receptor(s) will include experiments to determine possible association of 'Ia antigens' with the antigen specific receptor. With the nonimmunogenic polymers, we will determine whether immune suppressor (Is) genes control unresponsiveness, and whether 'gene complementation' might lead to responsiveness. Affinity chromatography techniques will be employed to isolate and characterize antigen-specific suppressor and helper factors as well as 'T cell receptors.' Reinvestigations of responses of mice of H-2s haplotype against GAT10, and studies on the effect of preparation and treatment of the polymers on responses will be undertaken. The B-cell mitogenic activity (mice) of sequential polymers will be investigated to determine whether these polymers are 'specific' or 'polyclonal' mitogens. The antibody produced in some of the 'genetically controlled' systems will be isolated and studied for possible homogeneity properties. The preparation of anti-idiotypic antibodies will be undertaken.

24. IMMUNE RESPONSE GENE REGULATION OF THE IMMUNE RESPONSE IN VITRO

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The cellular expression of immune response (Ir) gene function was studied in an in vitro system of primary antibody responses to the TNP conjugates of (T,G)-A- -L and (H,G)-A- -L. These responses require the participation of T cells and accessory cells as well as B cells, and are under the control of genes mapped to the K or I-A subregions of the H-2 complex. It was demonstrated that the function of accessory cells in responses to TNP-(T,G)-A- -L and TNP-(H,G)-A- -L is under the control of genes which also map to K or I-A. In contrast, both B cells and T cells from nonresponder H-2a strains to TNP-(T,G)-A- -L are competent in supporting primary antibody responses to this antigen, and therefore do not appear to express the Ir gene defect present in these strains. Subsequently, in vitro augmented primary responses to TNP-nuclease (TNP-NASE) have been established and documented to be under the control of H-2 linked Ir gene(s). For these responses as well, accessory cell function was shown to be under Ir gene control. Through the use of intra-H-2 recombinant strains, the Ir gene(s) controlling responsiveness to TNP-NASE were shown to map either to I-B or to complementing genes in subregions including at least I-A and I-E/C.

BIBLIOGRAPHIC REFERENCES: Singer, A., Hathcock, K. S., and Hodes, R. J.: MHC restricted helper T cell recognition of accessory cells but not B cells. In Unanue, E. and Rosenthal, A. (Eds.): Macrophage Regulation of Immunity. New York Academic Press, 1980, pp. 265-275. Singer, A., Hathcock, K. S., and Hodes, R. J.: Cellular and genetic control of antibody responses. V. Helper T cell recognition of H-2 determinants on accessory cells but not B cells. J. Exp. Med. 149: 1208-1226, 1979.

25. RECESSIVE GENES CONTROLLING IMMUNOCOMPETENCE

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The majority of inbred mice (29 out of 31 tested) are immunologically unresponsive to a single injection of adjuvant-free beta-D-galactosidase (Z). Two genetic loci, one dominant, Ir-Z1, the other recessive, ir-Z2, controlling this unresponsiveness have recently been found. These genes segregate as autosomal single loci and are not closely linked to either the H-2 or the Ig-1 region. It is proposed to study the function of Ir-Z genes at the genetic and cellular level. For this, congenic and co-isogenic strains carrying either Ir-Z1 or ir-Z2 will be constructed against a C57BL/6J genetic background. Subsequently, cell-to-cell interactions will be studied by adoptive transfer experiments and by in vitro cultures. The methdology available for studying immune responsiveness to Z is such that activating antibody can be detected at the single molecule level and antigen-binding cells can be easily enumerated. The objective is to establish the type of cell in which the products of the Ir-Z genes are expressed and ultimately, to isolate and characterize the gene products.

BIBLIOGRAPHIC REFERENCES: de Macario, E.C., Ellis, J., Guzman, R. and Rotman, B., 'Antibody-mediated Activation of a Defective beta-D-Galactosidase: Dimeric Form of the Activatable Mutant Enzyme. Proc. Natl. Acad. Sci. USA 75, 720-724, 1978.

26. GENETIC CONTROL OF THE IMMUNE RESPONSE

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Experiments are in progress to determine the species distribution of an Ia antigenic specificity defined by a monoclonal antibody designated H76. The Ir-2 immune response gene of mice is under investigation to determine whether it controls a population of suppressor cells. The gene product of the Svp locus in rats is being studied to determine whether it controls an immunosuppressive protein.

BIBLIOGRAPHIC REFERENCES: Gasser, David L., Winters, Alleen B., Haas, Jean B., McKearn, Thomas J. and Kennett, Roger H.: Monoclonal antibody directed to a B-cell antigen present in rats, mice, and humans. Proc. Natl. Acad. Sci. USA, Vol. 76, No.9, pp. 4636-4640, September 1979, Immunology.

27. GENETIC CONTROL OF THE MOUSE REPERTOIRE

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The objective of this proposal is to determine if genes located in the major histocompatibility system of the mouse regulate the expression of the B cell repertoire and how the

expression of the B cell clonotypes might change during ontogenic development. For this purpose, antigens known to be under Ir gene control, such as synthetic polymers of amino acids with a limited number of determinants will be used to study the extent and diversity of clonotype expression in responder and non-responder mice.

To study the control of expression of the murine B cell repertoire, the potential repertoire of an inbred strain must first be determined. Analysis of both the frequency of B cells responsive to the polymer under Ir gene control and the number of clonotypes involved in the response permits an estimate of the average representation of individual clonotypes within the repertoire. Precursor cells of defined specificity can be analyzed and enumerated when the combined techniques of adoptive cell transfer of limiting number of cells and the in vitro fragment culture system are combined.

Homogeneous antibody products obtained from hybrid cell lines resulting from the fusion of specific antibody producing cells and plasmacytoma lines will be used to obtain antisera against idiotypic determinants. The anti-idiotype sera raised will be used to examine the polymer-specific monoclonal responses and to characterize the B cell repertoire and non-responder mice.

28. GENETIC CONTROL OF NEOPLASMS IN RATS

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The purpose of this project is to examine genetic factors in rats that control immune responses and susceptibility to tumors induced by oncornaviruses. Humoral and cellular responses to viral and to other antigens will be measured by radioimmunoassays. These studies will include the amount and immunoglobulin class of antibodies and the amount and type of immune cells. Measurements will be made both during tumor growth and following immunization with virus or viral polypeptides. Genetic controls of responses will be related to the major histocompatibility (RT1) locus and to other phenotypic traits. Whether the quantity of virus released by tumor cells or the levels of virus replication differ in susceptible and resistant strains will also be examined. We plan to identify and amplify mechanisms of tumor resistance.

BIBLIOGRAPHIC REFERENCES: Jones, J., Jensen, F., and Feldman, J. Genetic Control of Immune Responses to Moloney Leukemia Virus in Rats. J. Natl. Cancer Inst. 60, 1467 (1978). Leung, K., Jones, J. and Feldman, J. Tumor Associated Antigens of Rat Moloney Sarcoma Cells. I. Cell surface antigens. J. Immunol. 121, 1836 (1978).

29. GENETIC CONTROL OF RESISTANCE AND IMMUNITY TO P815

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This application is for funds to accomplish: Specific aim 1 - to determine whether or not histocompatible tumor resistance to P815 segregates with the H-2b histocompatibility allele in the backcross DBA/2 x B6D2F1; and specific aim 4 - to generate congenic lines on a DBA/2 background selected by the criterion of histocompatible resistance to P815 based on survival.

30. IMMUNOLOGICAL ASPECTS OF AGING

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Research on the immunological aspects of aging deals with genetic factors in survival and genetic factors linked to MHC which control the immune deficiencies associated with aging. These genetic factors can be modified by dietary manipulation (hypocaloric diets). T cell deficiencies have been found in aging mice and humans. One important mechanism to explain it is the decreased response of spleen cells of aged mice to TCGF. In congenic strains of mice, H-2K and H-2b influence long-term survival in mice. Our results support the concept that one dose of the survival gene H-2b in mice is sufficient to improve survival. We will also study the effect of other genetic systems (not encoded by MHC) on survival. Ly subset profiles will be compared at different ages and the Ly1 and Ly2 alleles will be used as markers for survival in different genetic combinations. We have found that a possible mechanism to explain delayed tumor onset in mice with restricted diet is endocrinological, since these mice have delayed pregnancies. We will attempt to simulate pregnancies with Silastic implants with progesterone and estradiol 17beta. These mice and control mice will be studied by Ly subsets, onset of tumors and survival.

31. REGULATION OF IMMUNE RESPONSES

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OBJECTIVES: (a) To determine the part played by active suppressor mechanisms in tolerance to neonatally administered proteins. (b) To investigate idiotype-specific control mechanisms in the regulation of immune responses.

APPROACH: (a) CBA mice are made tolerant to BSA by 2 injections of 10 micrograms IP, at 1-3 and 7-10 days after birth. The presence of active suppressor cells in these mice is assessed in 2 ways: by cocultivating their cells with normal adult-spleen cells in BSA-challenged adult irradiated mice, by challenging them with a linked antigen complex containing both BSA and F gamma G, suppression being diagnosed as a depressed response to F gamma G. (b) The ability of T cells to collaborate with B cells from the same individual mouse has been compared with their power to collaborate with B cells from other, syngeneic individuals. Further experiments designed to show similar individual-specific regulatory or preference events are in progress, e.g., the effect of 'preimmunizing' animals with primed cells from another mouse, followed by a later transfer of (frozen then thawed) cells from the same mouse. The ultimate aim is to identify anti-idiotype regulatory mechanisms which may develop within a single animal as it undergoes an immune response.

PROGRESS: (a) The thymus appears to be a potent source of suppressor cells in neonatally tolerized mice. (b) T cell cooperate better with B cells from the same (primed) individual than with B cells from another individual, as reported in Eur. J. Immunol. (1978), in press.

32. MECHANISM OF B-HAPLOTYPE INFLUENCE ON GENERAL IMMUNE RESPONSE OF CHICKENS

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OBJECTIVE: Determine the mechanism by which the B-haplotype genes in chickens may be determining a general immune response that causes regression of virus induced tumors.

APPROACH: Line 151(5) inbred chickens have been mated to males of other inbred or selected lines with 7 different B-alleles. Congenic chickens that differ for the B-haplotype genes, but are randomly similar for all other genes, will be tested for several general immune bioassays that have been linked to the major histocompatibility complex in mice, other mammals or chickens. The bioassays may include hemolytic complement level, natural killer cell response, lymphocyte proliferation in graft-vs.-host response and control of hemolytic plaque-forming-cell response to heterologous erythrocytes. If differences in an assay are attributable to B-haplotype, experiments to correlate function in that assay with tumor resistance will be performed. Highly specific antisera will be developed to B-antigens shown to influence general immune function using hybridoma antibody technology. These sera will be used to quantitate and/or separate cells using a fluorescence activated cell sorter.

33. CELLULAR IMMUNOGENETIC CONTROL OF IN VITRO ALLOGRAFT IMMUNITY

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This research has investigated the nature of the cellular and genetic control of the in vitro generation of cytotoxic T lymphocytes. Specifically, we have investigated the role and nature of non-serologically determined target antigens in T-cell-mediated cytolysis, the role and genetic nature of proliferative and non-proliferative CML-helper signals in the induction of

cytotoxic T cells. We have also investigated the cellular nature of CML stimulator populations and cell-cell interactions among CML responder cells. Recently we have demonstrated that rabbit anti-mouse brain serum, a commonly used anti-murine T cell reagent, is mitogenic for mouse spleen cells. These studies have major implications in the fields of transplantation and tumor immunity

B. STRUCTURE AND FUNCTION OF GENES AND THEIR IMMUNOGLOBULINS

34. ALLELIC EXCLUSION OF IMMUNOGLOBULIN HEAVY CHAIN GENES

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During B-cell differentiation, two immunoglobulin heavy chain gene segments, V(variable) and C(constant), are rearranged into the functional gene. Allelic exclusion, or the expression of only one of two heavy chain constant region alleles present in a given lymphocyte, may be due to the exclusive translocation of only one of the homologous chromosomes. Alternatively, both chromosomes might be translocated: one 'abortively' and the other correctly joining V and C gene segments. We propose to investigate the molecular mechanisms of allelic exclusion in an (NZB x BALB)F1 mouse, heterozygous for the c alpha locus. Initially, we will examine DNA isolated from an F1 myeloma for both the normal translocation and any 'abortive' events. The restriction endonuclease digestion patterns of the parental DNAs differ, allowing determination of the origin of all C alpha-containing fragments. To exclude the possibility that 'abortive' events are due to myeloma aneuploidy, we will extend the analysis to IgA-producing diploid cells isolated from the lymphoid organ, Peyer's Patches. The cells will be fractionated by IgA allotype into two sub-populations: one producing IgA of maternal origin and the other of paternal origin. Genomic clones, constructed from both populations will be analyzed to determine if the non-expressed allele typically undergoes rearrangement during development.

35. ISOLATION AND ANALYSIS OF IMMUNOGLOBULIN GENES

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The objectives of this program are: 1. To clone and determine the structure of immunoglobulin light and heavy chain genes; 2. to establish the relationship of DNA rearrangements to the activation of immunoglobulin gene expression; 3. to determine the role of DNA rearrangements in immunoglobulin class switching.

Significant progress has been made towards these objectives. We have now constructed cloned genomic libraries from MOPC 21 myeloma cells and isolated the complete kappa light chain and gamma 1 heavy chain genes to complement our studies on the trancription and processing of the nuclear RNA precursors to MOPC 21 kappa and gamma 1 mRNA. We have also collaborated with Dr. Susumu Tonegawa on the cloning and characterization of MOPC 21 gamma 1 C-region heavy chain genes. We are using these cloned heavy and light chain genes to define the complete kappa and gamma 1 transcription units (including the signals for the origin and termination of transcription) and to further delineate the nature and order of nuclear RNA splicing steps in relation to specific RNA processing intermediates.

In collaboration with Dr. Lee Hood, we have recently isolated and characterized the immunoglobulin mu heavy chain C-region gene segment. This gene is now being used to study IgM gene expression and transitions in early B-cell development.

BIBLIOGRAPHIC REFERENCES: Fahey, J.L.: Principles of immunology with reference to immunotherapy. Immunotherapy of Human Cancer, Raven Press, New York, p. 31-39, 1978. Julliard, G.J.F., Boyer, P.J.J., and Yamashiro, C.H.: A Phase I study of active specific intralymphatic immunotherapy (ASILI). Cancer 41: 2215-2225, June 1978.

36. STUDIES OF RABBIT IMMUNOGLOBULIN ALLOTYPE GENES

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The organization and expression of rabbit immunoglobulin kappa chain constant region genes will be investigated by the use of cloning and nucleic acid hybridization techniques. Messenger RNA molecules coding for the region of the kappa light chain will be isolated from antibody producing cells expressing each of the four group b allotypes, b4, b5, b6, and b9. The cDNA transcripts of these molecules will be cloned in bacterial plasmids or bacteriophages and used as probes to test for the presence of unexpected C-kappa genes in the DNA of rabbits which are 'homozygous' for one of the allotypes. In this manner we hope to determine if the group b allotypes represent alleles at a single locus, or a closely linked family of duplicated genes with a polymorphic control mechanism which mimics a Mendelian pattern of segregation. Additional experiments with these probes and nucleic acid sequencing techniques should help to characterize the postulated control mechanism. The results of these experiments should have substantial significance for the genetics of the immune system.

37. MOLECULAR REGULATION OF IDIOTYPE IMMUNE RESPONSIVENESS

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Instead of myeloma models, we propose to study an idiotypic model of immunoglobulin gene expression. This model should more accurately reflect in vivo events and has known genetics. The model is based on the post-immunization production in all individuals within a mouse strain of antibody containing major idiotypic determinants which cross-react. By making idiotypically positive hybridomas we can purify Ig-mRNA, synthesize cDNA, and create idiotypic clones.

Variable region cDNA from these clones will be used as hybridization probes in Southern blots to study: 1) if the major idiotype is represented by a germline gene, 2) if a family of such genes exist, and 3) how these genes are rearranged in going from germline to expressed (as examined in the genomic DNA of the hybridomas).

From the above data, various genomic pieces will be selected to be cloned into Charon 4A lambda phage vectors. These clones will be restriction mapped and selected pieces sequenced. From these sequences and from those of the v-region cDNA's we hope to learn: 1) how families of v-genes vary in their sequences and how these variations relate to idiotypes, 2) what causes a certain member of a family to be expressed, 3) how to define idiotypes at the DNA level, and 4) how somatic mutations are involved in antibody diversity.

38. IMMUNOREGULATION OF ANTIBODY SYNTHESIS

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Antibodies specific for the idiotype(s) of anti-(T,G)-A- -L antibodies of two different mouse strains (C57BL/10Sn and C3H.SW) have been obtained. The idiotype(s) of these two strains are distinct and the anti-(T,G)-A- -L antibodies of the majority of individuals of each strain express identical or cross-reactive idiotype(s). Since the ability of mice to respond to (T,G)-A- -L is under the control of H-2 linked Ir genes, these anti-idiotype antibodies are being used in conjunction with radioimmunoassay of the binding of (T,G)-A- -L to anti-(T,G)-A- -L antibodies as well as both in vitro and in vivo humoral response assays to evaluate the cellular and genetic control of idiotype expression as well as the role of idiotype-bearing molecules in regulation of the immune response. T lymphocytes express anti-(T,G)-A--L idiotype(s) identical to or cross-reactive with those found on anti-(T,G)-A- -L antibodies of the same strain. Expression of similar or identical idiotypes is found on both IgM and IgG antibodies, and expression of these idiotypes

is controlled (at least in part) by genes linked to the heavy chain allotype locus.

BIBLIOGRAPHIC REFERENCES: Pincus, S. H., Singer, A., Hodes, R. J., and Dickler, H. B.: Anti-(T,G)-A- -L idiotypes: Initial studies of genetic control and cellular expression. In Pernis, B. and Vogel, H. (Eds.): Cells of Immunoglobulin Synthesis. New York, New York, Academic Press, 1979, pp. 371-380.

39. HIDDEN IMMUNOGLOBULIN ALLOTYPES IN MICE

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Reports of the transient appearance of allotypes not normally detectable (hidden) and not anticipated from genetic data have challenged the notion that allotypes are products of allelic structural genes. We have obtained evidence that a thymus-dependent mechanism may normally keep these allotypes hidden. To study this mechanism we are using mutant athymic mice.

BIBLIOGRAPHIC REFERENCES: Antigen-dependent stimulation of idiotype positive immunoglobulins lacking detectable antigen-binding activity. Enghofer, E., Bosma, M. and Glaudemans, C.P. Fed. Proc. 4191 (abstract), 1979. Regulation of a small family of VH genes. Enghofer, E. and Bosma, M.J., In: Journal of Supramolecular Structure 9: Suppl. 3 (abstract) ed. C.F. Fox, A.R. Liss Inc., New York (in press, 1979).

40. GENETIC MARKERS, LEUKEMOGENESIS, AND THYMIC FUNCTION

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The overall objectives largely involve analyzing the effects on the immune response of products of closely linked genes on chromosome 6 of the mouse which determine antibody light chains and the Lyt-2 and Lyt-3 T-cell surface alloantigens. Since our discovery that a kappa L-chain V-region polymorphism is closely linked to Lyt-2 and Lyt-3, we and others have described others, and it has been shown that kappa L-chain structural genes are located on chromosome 6. The structure and function of the Lyt-2 and Lyt-3 molecules are of great interest due to their presence on killer and suppressor but not helper T-cells, and because of the recent observation that killing activity of cytotoxic T-cells can be blocked by antisera and monoclonal antibodies which recognize Lyt-2 and/or Lyt-3. Work focuses upon isolation and characterization of myeloma kappa chains from congenic strains that we have constructed, genetic analyses of the role of the kappa locus in idiotype expression, isolation and characterization of monoclonal antibodies specific for Lyt-2 and Lyt-3, and structural characterization of the Lyt-2 and Lyt-3 molecules.

BIBLIOGRAPHIC REFERENCES: Jones, S.L., Cannon, L.E. and Gottlieb, P.D. Staphylococcal Protease Digestion of a Mouse lambda L Chain Yields a Large V-region Fragment Accessible to Direct Amino Acid Sequence Analysis. Molecular Immunology 16: 829-832 (1979). Durda, P.J., Boos, S.C. and Gottlieb, P.D. T100: A New Murine Cell Surface Glycoprotein Detected by Anti-Lyt-2.1 Serum. J. Immunol. 122: 1407 (1979).

41. GENETICS OF IMMUNOGLOBULINS IN PLASMACYTOMA CELLS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

C. OTHER BASIC IMMUNOGENETIC STUDIES

42. GENETIC ANALYSIS OF NORMAL AND MALIGNANT LYMPHOCYTES

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This research has the objective of combining the present knowledge of immunogenetics, as applied to the major histocompatibility complex, with the technology of separation of different lymphoid cells to further characterize the HLA linkage group. These studies will help to identify alloantigen systems restricted to T lymphocytes, monocytes and B lymphocytes. We intend to study several families in which we have found HLA recombination or HLA-GLO recombinations and HLA-C2 or HLA-Bf recombinations by the HLA-D polymorphism in order to map the complement loci. In another group of experiments utilizing cellular reagents we will investigate functional aspects of T lymphocytes (helper and suppression) following identification of alloantigens on resting T lymphocytes or on activated T lymphocytes. We hope to divide the HLA-D region into different subregions which may code for help or for suppression of the immune system.

BIBLIOGRAPHIC REFERENCES: DeWolf, W.C., Lange, P.H., Einarson, M., and Yunis, E.J.: HLA and testicular cancer. Nature (Lond), Vol. 277, No. 5693:216-217, 1979. DeWolf, W.C., Carroll, P.G., Yunis, E.J.: The genetics of PLT response. I. Methodology for optimal PLT discrimination. J. Immunol. 122:860, 1979.

43. IMMUNOGENETICS OF THE TLA REGION OF CHROMOSOME 17

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Qa:Tla genes specify glycoproteins that comprise approximately 45,000 (heavy chain) and 12,000 (beta 2M) dalton subunits and thus are structurally akin to H-2. The Qa:Tla members of this molecular family are distinguished by highly restricted developmental expression and by instances of null-allelism, modulation by antibody, and aberrant expression on malignant cells. We propose an extensive collaborative study of the basis of these distinctive features, combining immunogenetic and biochemical methods, with emphasis on microsequencing (primary structure). The first system chosen for detailed analysis is TL, isolated with a monoclonal TL.2 antibody of proven precipitating capacity. Full account is taken of reported failures to sequence TL (themselves a hint of significant H-2:TL structural differences); reasons for these failures are proposed, and matched with procedures to overcome or circumvent the biochemical problems implied. Major segments will be ordered by polypeptide fragmentation, disulfide cleavage, and N-terminal sequencing. Restricted proteolysis with trypsin and papain will be used to isolate surface-disposed H-chain pieces. Allied approaches include comparative peptide mapping to reveal disparity due to both TL haplotypic variation and aberrant TL expression by malignant cells (source material: thymocytes, including Tla-heterozygotes; and leukemia cells, including variant and non-variant lines immuno-selected for deletion of a critical Tla haplotype). A related goal is delineation of the Qa:Tla region in terms of assignment of Qa:Tl antigens throughout an ascertained set of approximately 45,000-dalton/beta 2M products. Working collaboration of our laboratories has been tested in a preliminary microsequencing exercise. The applicant's joint experience and facilities, in serological immunogenetics and relevant biochemistry, coupled with a fully operational hybridoma program, present an exceptional opportunity for a major commitment to the comparative chemistry of non-H-2 approximately 45,000 dalton/beta 2M glycoproteins of chromosome 17.

44. IMMUNOGENETICS OF SELF-IDENTIFICATION

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In the period reviewed, important steps were taken towards better understanding of sensory communication involving MHC genes: (a) With the mating preference assay, perception of MHC types was validated genetically by showing MHC mating bias among H-2 typed F2 segregants of crosses between MHC congenic mice. (B) A much simplified assay, with a differentially scented Y maze in which mice, trained by water deprivation and reward (reinforcement), distinguish MHC types, abolishes the complications of mating and vastly extends the prospects for detailed study of MHC sensory recognition. (c) Urines are as effective as mice as sources of MHC odors in the Y maze, which still further amplifies the opportunities for analysis. (d) MHC perception was confirmed in nearly 10,000 trials in the Y

maze, with inbred and F2 segregant mice and their urines as sources of odor.

Thus we are now in a highly favorable position to study, in the Y maze scented by mice or urines: (a) Recognition of the reinforced MHC type, when presented on different or variable genotypic backgrounds, as an index of the prominence of MHC sensory identity in relation to whatever total non-MHC sensory identity there may be. (b) Distinction of mice differing at subregions of the MHC and vicinity, as an index of genetic complexity of MHC recognition and to define least-complex systems for chemical study. (c) Distinction of H-2 mutant from nonmutant mice, a provocative probe for H-2 pleiotropism. (d) The chemistry of MHC-distinctive odors, probably multiple.

We have tentative evidence that the blocking of pregnancy by strange males (Bruce effect), which is subject to genetic variation in male potency and female susceptibility manifest in particular male-female genotypic combinations, is influenced by MHC types. We shall investigate whether genetic variation affecting induction of this neuro-endocrine response depends on concurrent sensory recognition of not only male pheromone but also the male's MHC type. If so, then another potential role for MHC perception in nature will be established.

45. IMMUNOGENICITY STUDIES WITH ANTIGEN-COATED LIPOSOMES

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DESCRIPTION: Artificial phospholipid vesicles (liposomes) will be coated with various cellular membrane antigens, in particular with purified viral antigens and with histocompatibility antigens. We want to determine the structural prerequisites for the induction of cellular or humoral immune reactions against membrane-bound antigens (antigen density, the mode of integration of the antigen into the membrane, hapten and carrier determinants). Liposomes will also be used to study the important questions of possible interactions of viral antigens and histocompatibility antigens in cellular membranes. Finally such liposomes, coated with viral antigens (virosomes), shall be used as a new type of vaccine. Such vaccines would be of great importance for human application since they do not contain the potentially dangerous nucleic acid of conventional vaccines.

46. IMMUNOGENETICS OF LY SYSTEMS

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Immunogenetic categorization of lymphocytes into sets and subsets separately programmed for coordinate expression of surface phenotype and function is crucial to much current immunologic research. Definition of the Ly123, Ly1 and Ly23 sets is only a beginning. Much formal investigation will be needed for even a bare description of the immune system in these new terms, because a single new system like Qa-1 can geometrically increase the details to be unraveled.

This grant renewal application centers on immunogenetic aspects and is geared to collaborative studies that should be particularly rewarding in this formative phase of the field. Technical additions to this laboratory, since the preceding award, are a unit for assay of immune functions in vitro and another for monoclonal antibody production by hybridomas, both now fully operational.

In addition to maintenance and expansion of our antiserum bank and tumor panel, the work outlined concerns: (a) Definition of new alloantigenic systems; unique features of the Ly-9 and 'U' (Qa-Tla region) systems are stressed. (b) Production of congenic mice for high potential systems. (c) Genetic linkage; the unmapped Ly-9 and Pca-1 loci head our list. (d) Phenotyping of cell sets in developmental sequence; with current emphasis on the Qa-1 (plus) and Qa-1 (minus) lineages. (e) Phenotyping of cooperative cell sets; with current emphasis on whether and how Lyl:Qa-1 (plus) and Lyl:Qa-1 (minus) sets collaborate in, and mediate, help. (f) Regulation; illustrated in the phenotyping of cell sets mediating feedback suppression of antibody production. (g) Selective T cell reconstitution of B mice; its evaluation as a general method for defining the ontogenetic rank and potential of defined cell sets. (h) Evaluation of flow cytometry and sorting for standardized particular applications; in collaboration with the Laboratory of Investigative Cytology. (i) Applications of hybridoma technology for monoclonal antibodies.

47. IMMUNOGENETIC MAPPING OF THE CELL SURFACE

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The objectives of this grant proposal are (1) to map, using serological techniques, the topographical arrangement of alloantigens on the cell surface of the mouse thymocyte and peripheral T lymphocyte, and (2) to investigate the induced rearrangement of these alloantigens called supramolecular 'repatterning' which is triggered by binding of an appropriate antibody to an alloantigenic site or, in the case of H-Y, by the hormone testosterone. We will initially study the physical interrelationships between the mouse alloantigens H-2D, H-2K, H-2L, Lyt-1, Lyt-2, Ly-5, TL, Thy-1, and Qa-2, and determine whether the relative positions of these cell surface components can be altered by binding with antibody. We will also determine whether such agents as colchicine and Cytochalasin B will inhibit alloantigenic repatterning. Finally, we will determine whether other agents besides antibody and testosterone will induce specific cell surface repatterning. Some genetic studies will also be performed. Methods to be used include the dual blocking assay, radio- or enzyme-immunoassays, antiserum production, hybridoma formation, cytotoxicity testing, and absorption procedures.

48. IMMUNOGENETICS OF NK-1(PLUS) NATURAL KILLER CELLS

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Spleen cells of untreated mice lyse BALB.RL male 1 (RL.1) leukemia cells in vitro. We have adopted this as a standard system for immunogenetic studies of natural killer (NK) cells. For our purposes we define the NK population as the cell set with RL.1 lytic capacity and surface phenotype Nk-1(plus). In preliminary work we have (1) established methods to enrich this NK cell set from heterogeneous populations; (2) adapted the Protein-A-SRBC rosette assay, which allows recovery and enrichment of both marker-positive and marker-negative viable cells, for use in the Nk-1 system; (3) established the Nk-1 phenotypes of 20 inbred strains; (4) determined that Nk-1 phenotypes are not related to levels of NK activity in different mouse strains, and (5) obtained evidence of a pro-Nk-1 cell set inducible for Nk-1 expression in vitro, permitting various uses of differentiative induction assays in the Nk-1 system. We propose (1) to define further the surface phenotype of this NK cell set in terms of expression of known immunogenetic surface markers; (2) to determine the differentiative origin of the NK cell set and its developmental relation to other cell sets; (3) to study the segregation of the Nk-1a (Nk-1.1) allele, map the Nk-1 locus, and to find alterative Nk-1 alleles and Nk-1 alloantigens; and (4) to determine whether C-type viral components are involved in NK activity in this model system. Thus we aim to determine the ontogeny of the Nk-1(plus) cell set and to elucidate the specificities of its RL.1 lytic activity.

49. GENETIC ASPECTS OF TUMOR-HOST IMMUNE RELATIONSHIP

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This research proposal involves four main components of a continuing study of the tumor-host immune relationship: 1. Plasmacytoma tumor specific immunity induced in vitro involves the generation of cytotoxic T cells specific to various tumor Ags. We will develop a series of continuous T cell lines reactive to different tumor antigens, including both MHC restricted and non-restricted lines, and of varying T cell subpopulations. These lines will then be used to characterize the T cell receptors, and the nature of tumor antigens recognized by these T cell populations. 2. Characterization and isolation of NK cells. A series of alloantisera and monoclonal antibodies specific for NK cells will be developed and used to isolate NK cells from a variety of

different sources using flow cytometry. The genetic controls of NK reactivity will be further assessed, and congenic lines of mice developed. 3. A series of T cell lymphomas will be characterized extensively for cell surface components with a wide range of hetero-, allo-, and hybridoma derived reagents. Such T cell lines will then be functionally studied as models of specific T cell subpopulations. 4. The series of T cell lymphomas and a variety of other tumors will be extensively analyzed with allo- and hybridoma derived reagents of various anti-H2 specificities, in order to assess the concept that tumor specific antigens may be inappropriately expressed normal MHC coded cell surface molecules.

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50. T CELL DEVELOPMENT - IMMUNOGENETICS, DEFECTS, THERAPY

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Proportions ('profiles') of the three main mouse T cell sets, Ly123:Ly1:Ly23, are characteristically distored in immune-deviant hairless (hr) mutant mice (increased Ly123: decreased Ly1), and after thymectomy (decreased Ly123: increased Ly1).

We propose to define these models further, especially in terms of Qa-1 subsets (because of the evident bearing of Qa-1 on suppression and immune regulation), in company with nu/nu athymic mice. Equal weight is given to (a) elucidating various bases of immunodeficiency and deviation, genetic and non-genetic, and (b) evaluating therapeutic agents - thymopoietin-TP5, ubiquitin, TCGF - in deficient, deviant and normal mice. In conjunction with tests of immune function in vitro, these immunogentic studies are intended as paradigms for investigating the nature and therapy of human disorders of immunity.

New studies, with pleiotropic-mutant W mice, aided by the Ly-5 marker system, promise to extend immunogenetic analysis to pre-lymphocytic stem sets and to identify the reputed T cell said to collaborate in erythropoiesis.

Special emphasis is placed on (a) derivation of mouse strains and stocks of Tlaa:hr genotype, to allow Qa-1 phenotyping of hr/hr and plus/hr mice, (b) determining whether hr is fully recessive, or causes a minor syndrome in heterozygotes (special relevance to man), for which purpose congenic plus/plus mice are being bred; and (c) study of ranges of normal profile variation and profile change with age in the species.

51. CHEMICAL GENETIC AND CELLULAR ASPECTS OF IMMUNOGENICITY

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The studies described in this proposal continue our work on the immunogenicity of synthetic polypeptide antigens and have expanded into an integrated chemical, genetic and cellular approach to our long-term objectives of explaining in chemical terms how an antigen acts to elicit an antibody response and what the molecular basis of the genetically controlled differences in the response are. Studies on the genetic control of the antibody response have led to free development of a polygenic model for the control of antibody response. There is a sex influence on the immune response: the amount of antibody formed by females is larger and more heterogeneous than that formed by males. We are presently trying to determine whether this sex influence is genetic and/or hormonal. The maternal-fetal interaction can markedly affect the ability of the fetus to respond to an antigenic stimulus: immunization of the mother enhances the ability of the offspring to form antibody. This effect is being studied to determine the mechanism by which it occurs.

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52. CELLULAR AND GENETIC ASPECTS OF ANTI-TUMOR IMMUNITY

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The immune system, with its complex network of antigen driven, and non-antigen driven pathways, is quite dynamic with regards to the nature and type of elicited responses. The final products of the numerous available mechanisms can function either synergistically or antagonistically with respect to one another. In response to stimulation with tumor antigens, either effector (rejection) or suppressor (progression) responses can eventually dominate. Thus, for many reasons, it is necessary to consider characteristics of tumors, the immunologic status of the host and environmental influences (both to the host and the tumor) concomitantly in any valid and complete study. In this context, our laboratory investigates questions related to the immunological balance which exists between a host and its tumor. These include a determination as to whether Langerhans cells are involved in the induction of tumor specific suppressor T cells by epidermal ultraviolet light exposures. In addition, we are currently evaluating the interrelationship which exists between the MHC and ultraviolet light mediated immunoregulatory influences as well as the heterogeneity of immunologic regulatory mechanisms which influence tumor progression. Tumor cell cloning is proving to be a valuable tool for the study of tumor composition. Clones (the product of single tumor cells) are proving to be homogeneous with regards to tumor antigen expression and heterogeneous with regards to tumorigenic properties when compared to the parental tumor. By employing regressor and progressor clones derived from a single tumor which are cross-immunogenic, we hope to develop a better understanding of the biochemical and immunological basis of tumor progression in the face of antigenic disparity.

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53. IMMUNOGENETICS OF TUMOR RELATED ALLOANTIGENS

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The host range of and host-cell susceptibility to the leukosis-sarcoma virus is dependent on specific interactions between genetically controlled properties of the viral envelope and the host-cell plasma membrane. The cell membrane 'receptor' may act as an inherited alloantigen and be detectable by existing serological methods. In an effort to produce alloantibodies specific for receptors controlling susceptibility to the viral subgroups A,B, and C, chickens of known receptor genotype and compatible erythrocyte alloantigens at 11 other loci will be cross-transfused. The resulting antisera will be analyzed using lines of chickens fixed for different combinations of A,B, and C receptors and of known alloantigen constitution.

Another primary objective is to investigate the substructure of the erythrocyte alloantigen-determining portion of the B histocompatibility complex. The significance of this investigation rests on the finding that certain B haplotypes either resist specific tumor formation or are very effective in regressing them. Currently, several recombinants within the B complex are under investigation for effects of substructure components.

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with B major histocompatibility complex. Immunogenetics 10: 169-174.

54. THE GIX SYSTEM - IMMUNOGENETICS OF ENDOGENOUS MULV

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This is part of a broader project. A summary of this subproject is not available.

55. IMMUNOGENETICS IN HERPES ZOSTER

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This is part of a broader project. A summary of this subproject is not available.

III. MAJOR HISTOCOMPATIBILITY COMPLEXES AND CANCER

A. SPECIFIC DISEASES ASSOCIATED WITH THE HLA COMPLEX

56. HLA-B LOCUS BLANKS AND DISEASE PROGRESSION IN MELANOMA PATIENTS

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OBJECTIVE: The aim of this work is to verify whether an increasing number of HLA-B locus blanks may be associated or not with disease progression in melanoma patients, as suggested by us in our recent study on 'HLA antigens in malignant melanoma patients' (in press).

APPROACH: Two groups, each of 30 Caucasoid patients suffering from a first stage of melanoma, will be HLA typed before surgical removal of tumor and regularly at two month intervals for a period of two years: one group showing blank(s) at HLA-B locus, not related to the homozygosity or to an unidentified allelic residue; the other not carrying blank(s) at the HLA-B locus, both at the first HLA typing.

The lymphocytotoxic test will be used.

57. RELATIONSHIPS OF SOLUBLE TUMOR AND HLA ANTIGENS IN BLOOD SERUM FROM MELANOMA PATIENTS

Zanussi, C., Scorza, R., Sabbadini, M. G., Fabio, G., Bonara, P., Vanoli, M., Pettenati, C., Barbaglio, G., Universita degli Studi di Milano, Istituto di Clinica Medica IV, *Via F. Sforza 35, 20122 Milan, Italy*

The aim of our work is to investigate the presence of soluble HLA-TAA antigens in peripheral blood sera from melanoma patients and the presence of HLA alloantigens not found on peripheral blood lymphocytes (PBL) from the same subjects. The final purpose is to isolate and to purify such antigens and to employ them in immunochemical investigations and in functional studies in vitro to clarify their role in the specific immunosuppression of cancer patients. QAE-Sephadex 0.3 M NaCl serum fractions containing HLA soluble antigens were obtained from 7 control subjects and 12 melanoma patients. Each fraction was characterized by means of the cytotoxicity on relevant autologous lymphocytes (PBL in the case of HLA-A and B specificities; B lymphocytes for the HLA-DrW antigens) and the ID50 for each antigen was determined. Four control fractions and 7 fractions obtained from melanoma patients were absorbed in vitro with autologous lymphocytes which were previously allowed to shed in vitro surface molecules eventually absorbed in vivo. Such an absorption produced, in the case of melanoma but not of control fractions, a strong although not complete removal of the inhibition exerted by the unabsorbed fraction in the cytotoxicity inhibition test. Such a variation was observed only for soluble antigens coded by HLA-B and Dr loci and, in the single patient, only B and/or DrW soluble antigen was affected.

One soluble HLA-B antigen and 2 soluble DrW specificities obtained from 2 melanoma sera were further purified by means of specific immunoabsorption by anti-HLA coated CNBr-activated Sepharose columns. The purified antigens were employed to raise anti-HLA heterologous rabbit antisera that would be employed in absorption and cytotoxicity tests with lymphocytes and melanoblastoma autologous and homologous cells.

58. HL-A IN FAMILIAL MALIGNANT MELANOMA - GENETICS OF SKIN CANCER

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The following investigations are in progress: (1) Studies of HL-A in six families in which more than one member has had malignant melanoma have been very rewarding. It is proposed to continue this study in a further sixteen families and hence possibly further define a sub-group-at-risk for malignant melanoma. (2) A genetic diathesis termed 'Celticity' has been established in skin cancer in Australians and in residents of Northern America. HL-A phenotyping as well as other genetic markers are being used in early severe cases of basal cell and squamous cell carcinoma and the role of other factors such as wind, infrared and ultraviolet exposure and humidity are being investigated. Controls consist of age and sex matched patients without skin cancer.

59. ARE HUMAN MELANOMA ASSOCIATED ANTIGENS MODULATED HISTOCOMPATIBILITY ANTIGENS?

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The major aim of this study is to gain a better understanding of the molecular level of the role played by cell surface molecules in the development of cancer in man and the immune protection against neoplastic disease. To this end, efforts will be made to assess 1) whether human melanoma associated antigens are chemically modulated human histocompatibility antigens, i.e., self-recognition markers, and 2) whether there is any correlation between the chemical structure and the biological function of these tumor associated antigens.

60. IMMUNOCHEMICAL CHARACTERIZATION OF ANTIGENS IN MELANOMA

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Cultured melanoma cells express DR antigens, a set of histocompatibility antigens which have restricted tissue distribution and share several biological molecular properties with murine Ia-E subregion antigens. Xenoantisera to human melanoma associated antigens have been prepared and characterized with serological assays. These xenoantisera have been used to define the biological and molecular profile of human melanoma associated antigens.

61. EPIDEMIOLOGIC AND HLA STUDY OF LEUKEMIA

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The major objective of this research is to study the postulated association between CLL and chronic antigenic stimulation (related to infections, inflammatory processes and autoimmune diseases) by means of medical chart review and interview in a case-control study. We are also exploring the postulated increased familial incidence of CLL and other lymphoproliferative malignancies. We are examining possible associations of CLL with occupation, smoking, alcohol use, immunizations and operative procedures involving the lymphoproliferative system (tonsillectomy, splenectomy, etc.) in order to develop additional etiologic hypotheses.

A medical record room search of all Baltimore SMSA hospitals is one of the prime sources for ascertaining cases. Physicians have reviewed 339 charts at 2 of the 3 major university hospitals and at 2 of the largest community hospitals where a large proportion of CLL cases are expected to have been seen. At present, this has yielded 121 cases diagnosed as CLL. Actually, 11/121 (9%) cases called CLL did not meet the criteria for CLL as established by the NCI CLL/Myeloma Task

Force because of absolute lymphocyte counts less than 15,000/mm3. 28/121 (23%) of cases called CLL are probably cases of well-differentiated lymphocytic lymphoma.

Each case is matched with two controls with any malignancy other than a lymphoproliferative neoplasm and two controls with any non-malignant disease. The matching variables include age (within 5 years), sex, race, date of diagnostic admission and hospital of admission.

We have just begun the interview and blood-drawing portion of the study. We hope to identify differences in HLA types between patients with CLL and controls. We will then attempt to correlate these differences with epidemiologic and clinical characteristics of the various subgroups of our population of cases and controls.

62. DETECTION BY CML OF HISTOCOMPATIBILITY ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

63. HUMAN LEUKEMIA AND LYMPHOMA - LYMPHOCYTE MEMBRANE ALLOANTIGENS AND MEMBRANE MARKERS (ABBREV)

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OBJECTIVE: Investigation of the serological behavior of lymphoid and/or blastic cells from leukemic patients with human alloantisera, especially those containing anti-HLA-DR antibodies. Correlation with other membrane markers, leukemia or lymphoma types and clinical outcome.

APPROACH: Peripheral blood lymphocytes and/or leucoblasts (and, when available, lymphoid cells from lymph nodes, bone marrow, etc.) from both acute and chronic leukemia and from various lymphomas are studied in terms of HLA-A,B,DR typing, by complement-dependent microlymphocytotoxicity test; T or B membrane markers, including surface immunoglobulins, human T lymphocyte antigen (HLTA), receptors for Fc fragments, complement, mouse red blood cells and sheep red blood cells; their ability to detect low levels of various antibodies in human alloantisera.

PROGRESS: Preliminary results suggest that there does not seem to be any striking difference between the HLA-DR antigen frequencies of the different types of leukemic patients and healthy blood donors. Studies comparing the expressivity of these antigens and the other membrane markers in the different types of leukemia are in progress.

64. RELATION OF HLA ANTIGENS AND LYMPHOCYTE FUNCTION TESTS TO TUMOR SUSCEPTIBILITY AND CLINICAL COURSE IN E. AFRICAN BL AND NPC (ABBREV)

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OBJECTIVE: (a) To determine whether there is any link between certain alleles of the major histocompatibility complex (particularly of the HLA DR locus) and susceptibility to Epstein-Barr virus (EBV), related tumors in East African subjects. (b) To measure blood lymphocyte responses to a range of antigenic and mitogenic stimuli in serial specimens from the same donors.

Answers to (a) may help to identify individuals at high risk of developing these tumors, and may provide some clues as to the nature of the interaction between EBV and the target cell. The object of (b) is to seek some definition, in quantitative terms, of the clinical phenomenon of 'host response' to these tumors.

APPROACH: (a) HLA typing at the A, B, C and D loci will be carried out on cryopreserved lymphocytes from at least 75 East African patients with Burkitt's lymphoma (BL), 80 first order relatives (consisting of families in which multiple tumors have occurred and families of individual BL patients where specimens from both parents and siblings are available), at least 75 nasopharyngeal carcinoma (NPC) patients, and matched control subjects (including families) from the same geographical area will be examined. Standard tissue typing sera will be used as well as a panel of sera derived from East African women subsequent to pregnancy. (b) A new microassay developed by Dr. S. C. Knight and extensively validated on cryopreserved lymphocytes from the I.A.R.C. cell bank makes it possible to measure the response of cells from a single ampoule to a range of antigenic or mitogenic stimuli. These include phytohaemagglutinin (PHA) and mitomycin C blocked cells from lines such as U266BL (derived from human myeloma) and DAUDI (from BL).

65. HLA STUDIES IN PATIENTS WITH MYCOSIS FUNGOIDES

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This is part of a broader project. A summary of this subproject is not available.

66. IMMUNOGENETIC AND IMMUNOLOGICAL ASPECTS OF NASOPHARYNGEAL CARCINOMA (NPC)

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HL-A profiles associated with risk of development of NPC and with prognosis have now been established. The haplotype A2 BW46 (Sin 2) is associated with a relative risk (RR) of 1.97 and 2.29 in two studies of newly diagnosed NPC patients. Another haplotype, AW19 B17, is associated with a RR of 2.80 and 2.60 in the two studies. AW19 B17 and, to a lesser extent, A2 BW46 have been found to be associated with poor survival in a restrospective study. HL-A - DR locus antigen typing in NPC patients has begun. NPC patients showed a higher frequency of DR locus blank antigen than controls. A local DR antisera, AH21, showed higher reactivity among the patients than controls (RR equals 8.0). A prospective study of HL-A profiles and prognosis in NPC is in progress. A prospective study of HL-A, Epstein-Barr virus serology and development of NPC in a normal population has also been started.

67. HLA ANTIGENS IN PATIENTS WITH INTESTINAL POLYPS

Illeni, M. T., Clerici, E., Pellegris, G., Spinelli, P., Pizzetti, P., Pasquali, M., Istituto Nazionale per lo Studio e la Cura dei Tumori, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: Some genes involved in the pathogenesis of tumors are believed to be contained in the greater complex of histocompatibility, closely associated with the genes which control the expression of HLA antigens. An increased incidence of some HLA antigens in a given neoplastic form can thus indicate the existence of a 'linkage disequilibrium' of these genes with the genes involved in the pathogenesis of the neoplasm.

The phenomenon can be used clinically to select a high-risk population for a given tumor.

APPROACH: HLA typing, indicated in protocol 4/1 of 1976, is in progress. The lymphocytotoxicity test is used.

We will also look for correlations with blood group ABO, Rh, Lewis, the secretory nature of group-specific substances, as well as histological type. Familial studies will also be conducted.

PROGRESS: Fifty-two cases of non-familial single and multiple polyps have been typed up to now.

A highly significant increase was observed in the HLA-Bw22 antigen in the patients as compared to the controls (p equals 0.00006). (Translated)

68. HLA AND GENETIC MARKERS FOR TERATOCARCINOMA

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The objective of this proposal is to investigate the significantly increased HLA-Dw7 antigen frequency in patients with testicular teratocarcinoma. This will be done by critically analyzing and reconstructing the triangular relationship between the mouse major histocompatibility complex (H-2), the T/t complex, and teratocarcinoma, and then applying this information,

as a guide to look at genetic regions in linkage with HLA in hopes of defining genetic markers in association with teratocarcinoma. Experiments will be designed comparing a testicular teratocarcinoma population to a normal control population from the same geographical area in terms of: 1) Genetic markers in linkage with HLA (GLO, PGM3, Bf, C2 and HLA-DR) and 2) genetic markers expressed on spermatozoa. Because T/t complex alleles are expressed only on spermatozoa as the only normal adult tissue in mice and control the expression of a teratocarcinoma associated antigen on sperm (F9), a systematic investigation will be made for a T/t complex-like region near HLA (in the human), controlling the expression of genetic markers present on spermatozoa of teratocarcinoma patients. Considering the homology between the mouse and human major histocompatibility complex (H-2 and HLA), such a region in the human should be included within the same genetic areas as the other markers, i.e. between HLA-D and the centromere. The detection of spermatozoa-specific teratocarcinoma associated antigens will involve three different techniques: 1) Lymphocyte-spermatozoa primed lymphocyte typing (PLT); 2) two-dimensional protein electrophoresis of various components of spermatozoa and 3) screening of sera (i.e., HLA alloantisera, sera from teratocarcinoma patients) for anti-spermatozoa activity. We expect that the structural, genetic, and developmental association between teratocarcinoma, and T/t complex, and H-2 in the mouse, will help unravel the relationship between HLA-Dw7 and teratocarcinoma as well as provide an effort toward the definition of a T/t complex-like region in the human.

69. HL-A ANTIGENS IN WOMEN WITH TROPHOBLASTIC NEOPLASMS

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This is part of a broader project. A summary of this subproject is not available.

70. CHARACTERIZATION OF HUMAN HLA-D GENE PRODUCTS AND THEIR RELATION TO DISEASE SUSCEPTIBILITY

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The major objective of this project is to further our understanding of the mechanisms underlying the associations between genes in the major histocompatibility complex and various diseases, especially neoplastic disease.

It is my specific aim to identify and characterize those cell surface molecules coded for by the HLA-D locus which are associated with these diseases and to determine if there is a single molecule or a group of molecules associated with disease susceptibility.

In order to isolate and characterize the product of the MHC in man, our aim is to apply new techniques which have been established recently in this laboratory for use in the immune system. These techniques include: 1. The ability to produce large quantities of pure monospecific heteroantibodies directed against the HLA-D region products by somatic cell hybridization. 2. The possibility of separating polypeptide chains by two-dimensional gel electrophoresis leading to specific 2-D gel Ia profiles. These patterns will be compared in HLA-D homozygous lymphoblastoid cell lines, then in heterozygous cells, and in cells from patients with different diseases.

71. HLA AND DISEASE ASSOCIATIONS IN OTHER ETHNIC GROUPS

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This is part of a broader project. A summary of this subproject is not available.

B. SPECIFIC DISEASES ASSOCIATED WITH THE H-2 COMPLEX

72. IMMUNOLOGIC RESISTANCE AGAINST TYPE C RNA VIRUSES AND VIRUS INDUCED LYMPHOMAS IN H-2 CONGENIC C57BL MICE

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OBJECTIVE: The proposed project deals with oncogenesis by naturally occurring type C viruses of C57BL mice. The following questions are asked: 1) Which H-2 (sub)regions are responsible for the recently established H-2 effects on tumor incidence, virus titers, anti-virus antibody responses and anti-tumor cell antibody responses? 2) What is the aetiology of lymphomas in C57BL mice with (V positive) and without (V negative) milk transmission of B-tropic virus? 3) Which viral antigens are expressed at the tumor cell surface? 4) What is the specificity of anti-tumor cell antibodies in tumor bearing and non-tumor bearing V positive and V negative mice?

The C57BL mouse model offers the following advantages: a) C57BL mice have not been genetically selected for high lymphoma incidence. This increases the chance of detection of genetically determined resistance factors. b) The epidemiology of virus-induced lymphoma in C57BL mice is remarkable similar to that in wild mice. c) Congenic C57BL mouse strains are available which are genetically identical except for H-2. This offers a unique opportunity for analysis of already observed H-2 effects on tumor incidence and immune responses.

APPROACH: We will try to map H-2 effects on: lymphoma incidence, virus titers, antibody response against viral envelope antigens, antibody response against virus-induced tumors. In this study a panel of 13 H-2 congenic C57BL mouse strains will be used. In addition, the specificity of anti-tumor cell antibodies will be studied in correlation with the expression of virus-associated antigens at the surface of virus-induced lymphoma cells.

73. RELATIONSHIPS BETWEEN HISTOCOMPATIBILITY ANTIGENS AND TUMOR SPECIFIC TRANSPLANTATION ANTIGENS OF METHYLCHOLANTHRENE-INDUCED SARCOMAS

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OBJECTIVE: To isolate, biochemically characterize and compare the tumor specific transplantation antigens (TSTA) with the histocompatibility antigens of methylcholanthrene-induced murine fibrosarcomas.

APPROACH: Fibrosarcomas are induced in BALB/c, B10, B10.BR, B10.D2 and B10.M mice by subcutaneous application of methylcholanthrene. After 3-4 in vivo passages the tumors are then examined for the presence of TSTA by in vivo transplantation techniques, and for the expression of normal and abnormal MHC products by serological and biochemical methods. A panel of syngeneic and semisyngeneic anti-TSTA sera is being raised in appropriate hosts to produce reagents to be used in RIA against soluble, partially purified TSTA.

74. EXPRESSION OF H-2 ANTIGENS ON SJL/J TUMORS

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Spontaneous reticulum cell sarcoma (RCS) and methylcholanthrene (MCA)-induced tumors of SJL/J mice will be used as a model for the study of alterations of MHC transplantation antigens (loss or gain). Enriched and purified preparations of spontaneous and early in vivo passaged RCS tumor cells, as well as MCA-induced tumors will be examined, 'respectively' for H-2 expression using a panel of monoclonal antisera. Parameters such as the identity of the tissue source from which tumor cells are isolated and the degree of tumor progression at the time of isolation will also be investigated. Serological analyses to be used for H-2 antigenic characterization will be dye exclusion cytotoxicity, cytostasis, immunofluorescence, absorption analysis, and blocking test. Cell mediated lympholysis (CML)

studies will also be done. Biochemical characterization of the unexpected antigens, using immunoprecipitation and sequential immunoprecipitation in SDS-PAGE analysis, and 2-dimensional gel electrophoresis will provide data for comparison with normal H-2K or D region gene products. Additional support has been sought to examine the biological relevancy of all alterations which are noted. These studies therefore will give insight into the correlation between altered H-2 expression and the ultimate fate of a tumor cell population as well as the mechanisms by which such alterations might occur.

IV. HISTOCOMPATIBILITY GENES AND ANTIGENS

A. STRUCTURE AND FUNCTION OF GENES OF THE MAJOR HISTOCOMPATIBILITY COMPLEXES

75. CELL-CELL INTERACTIONS IN THE HUMAN IMMUNE RESPONSE

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In rodents, T cell recognition is usually restricted by the major histocompatibility complex (MHC), and for helper T cell induction in the mouse there is a requirement for antigen presentation by I region-compatible macrophage-like cells. We have investigated the genetics of human T helper cell recognition using DR/DW-typed donors. It was found that compatibility or semi-compatibility at the HLA-D region was essential, in analogy to the findings in the mouse. Attempts have been made to use this assay for the detection of human Ir genes, using T cells from 'responders' or 'non-responders' (to synthetic polypeptide antigens) and culturing these with macropahges purified from 'responder' or 'non-responder' donors. Preliminary results have been most interesting, as semi-allogeneic responder macrophages have induced non-responder T cells to yield helper cells, suggesting that immune response genes are also predominantly expressed in macrophages in human systems.

B cells from human blood, unlike those from the tonsil, have been notoriously difficult to culture with conventional antigens. As this may be due to the fact that they are in an inappropriate stage of their growth cycle, attempts have made to add growth factors, in the form of conditioned media, to cultures of human peripheral blood lymphocytes (PBL). Responses to sheep red cells have been induced in the presence of the appropriate helper factor and growth factor, but as yet the system is still not fully reproducible.

Mouse T cells cultured with high doses of antigen yield suppressor cells, which release suppressor factors. Experiments of this type have been performed with human PBL and potent suppressor factor has been obtained which inhibited responses of primed or unprimed mouse spleen cells. As in the mouse, suppressor factors may be partly purified by absorption and elution in antigen columns.

76. ISOLATION AND CLONING OF THE H-2 GENES FROM THE MOUSE MAJOR HISTOCOMPATIBILITY COMPLEX

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The overall objective of this research is to clone and isolate the complementary DNA sequences corresponding to one or more of the histocompatibility-2 (H-2) genes of the mouse. Since each of the messenger RNAs (mRNAs) for H-2K, H-2D, and H-2IA probably represents less than 1 percent of the total cellular mRNA, the plan to isolate sequences homologous to H-2 loci will be divided into two phases. The first phase is the preparation by well-established procedures of an mRNA fraction enriched for H-2 mRNA activity. In the second phase recombinant plasmids containing cDNA synthesized from the enriched RNA fraction are screened for the presence of H-2 sequences. This approach eliminates the requirement for extensive RNA purification since cloning serves as the final fractionation step. Highly specific immunological probes will be used to detect H-2 mRNA activity during the mRNA enrichment phase and the recombinant colony screening phase. Immunological probes that are already available to us are monoclonal antibodies to H-2Kk, H-2Db, and Ia determinants, as well as conventional anti-H-2 sera.

77. CLONING OF THE I REGION OF THE MOUSE MHC

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The I region of the mouse major histocompatibility complex contains genes encoding highly polymorphic cell surface glycoproteins known as the Ia antigens. Ia molecules may be the functional gene products of I region genes which control antigen specific immune responsiveness. Ia mRNAs can be identified by the immune precipitation of Ia antigenic material in an in vitro translation system. Differential nucleic acid hybridization techniques exploiting the differences in expression levels of Ia molecules on T and B lymphocytes and hybridization arrested translation will both be used as primary assays for the isolation and identification of Ia-cDNA clones. I propose to examine the genomic DNA sequences encoding the Ia antigens in order to determine the source of the biochemical, strain dependent polymorphism and possible antigenic specificities of its gene products.

These structural studies will be expanded to a functional analysis of the Ia genes for specific immune response function by transformation of appropriate cloned cell lines (and normal cells) with defined DNA sequences. These functional analyses yield the tantalizing possibility of the eventual genetic manipulation of the immune system in the understanding, treatment, and prevention of human disease.

78. STUDIES ON H-2 MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) MUTANTS (STRUCTURAL ANALYSIS AS A PROBE OF FUNCTION-STRUCTURE RELATIONSHIPS)

Nathenson, S. G., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Dept. of Microbiology & Immunology/Cell Biology, *1300 Morris Park Ave., Bronx, New York,* 10461, United States of America

The H-2 major histocompatibility complex (MHC) is a complex of genes vital to the system of immune recognition and reaction. Studies are proposed to determine the structural basis of the mutation in the K or D genes of the H-2 MHC which are responsible for histogenic and, in some cases, serological reactivity with the parent strains. Analysis of the mutant products will be carried out by tryptic peptide mapping to determine if differences exist. Localization of tryptic peptide differences will be made to specific CNBr fragments in order to localize the mutational site within the molecules. Sequence analysis from selected mutants will determine the nature of the molecular alteration of the mutation.

79. PRODUCTION AND ANALYSIS OF HISTOCOMPATIBILITY MUTANTS

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More than 50 lines of mice carrying histocompatibility mutations have been or are being developed in this laboratory. Such mutants have become increasingly useful in a wide range of immunogenetic and immunobiological research. We propose to establish and maintain the current lines, to add to this unique collection as new mutations are recovered, to identify and map the mutated genes insofar as is practical, to utilize the mutants in immunogenetic studies (our own and those of collaborators), and to ultimately make the completed and defined mutant lines available to other investigators.

We currently hold 20 H-2 and about 30 non-H-2 mutations. We plan to map the H-2 and non-H-2 mutations, characterize their effects on skin graft rejection and on resistance to certain transplantable tumors. We also plan to study mutant histocompatibility genes whose expression is multigenically determined and can be altered by changes in the genetic background of the graft donor. In addition to our own studies, we will systematically supply mutants to certain collaborators for their studies on

the mutants with respect to serological changes, in vitro cell mediated reactivity, structural analysis of the mutant gene products, and tumor immunology.

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80. GENETIC FINE STRUCTURE OF THE H-2 GENE COMPLEX

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The murine H-2 major histocompatibility complex of MHC controls a diverse set of products and traits whose definition is important to the understanding of fundamental genetic and immunologic mechanisms, as well as to clinically relevant research in transplantation and in resistance to neoplastic and other diseases. In the latter context, H-2 is an important experimental model for the human MHC, the HLA gene complex, because of the remarkable genetic and functional homology. The long-term objective of this research is to resolve, by genetic, biochemical and functional approaches, the multiple genes of the H-2 complex, in order to advance our understanding of the organization, evolution and regulation of the MHC genes. This research will focus particularly upon the genes of the internal I and S regions of the H-2 complex, whose products mediate macrophage-T lymphocyte-B lymphocyte regulatory interactions, and reactions of the complement pathways, respectively. Efforts will be made to detect and characterize new mutations and recombinations in order to improve H-2 genetic fine structure mapping and to provide new and useful stocks for further functional and biochemical studies. Progeny from mutagen-treated parents will be screened by mixed leucocyte reactions and with monoclonal antibodies to cellular alloantigens. Variants will be characterized serologically, biochemically and functionally, with emphasis on improved definition of the I-A, I-B, I-J, S and D regions.

81. GENOME ORGANIZATION OF MURINE MAJOR HISTOCOMPATIBILITY COMPLEX

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This study was initiated to determine the DNA sequence organization of the genes encoding the murine major histocompatibility complex and the mechanisms controlling the expression of these genes.

82. H-2 COMPLEX AND SUSCEPTIBILITY TO MAMMARY TUMOR VIRUS

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This investigation is concerned with the influence of the major histocompatibility complex (H-2) on susceptibility of mice to mouse mammary tumor virus (MuMTV). Various strains of mice congenic for the H-2 complex with a C57BL/10 or BALB/c background have been selected for testing. Preliminary studies with these congenics have established that they do not express MuMTV in their milks and that although low levels of MuMTV RNA can be detected in lactating mammary glands, no significant differences exist in the expression of endogenous MuMTV information. These strains of congenic mice are being inoculated with MuMTV from various sources. The concentrations of virions in the inocula are quantitated by nucleic acid hybridization assay. Virus replication in the infected hosts is being measured by radioimmunoassay for gp55, the major virus glycoprotein, in the milks of third lactation mice, and mice are also being observed for incidence of mammary tumors. These data should establish the influence of the H-2 complex on MuMTV susceptibility and the location of the gene(s) involved. In addition, techniques are being developed to study the cellular and humoral immune responses of these mice to virus antigens in order to elucidate the mechanisms involved in H-2 determined susceptibility to a virus-associated carcinoma.

BIBLIOGRAPHIC REFERENCES: Moore, D.H., Long, C.A., Vaidya, A.B., Sheffield, J.B., Dion, A.S., and Lasfargues, E.Y. Mammary Tumor Viruses. In: Advances in Cancer Research, Vol. 29, 1979.

83. STUDIES ON THE REGULATION AND CONTROL AND GENE STRUCTURE OF H-2 HISTOCOMPATIBILITY GLYCOPROTEINS

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The H-2 major histocompatibility complex (MHC) is a complex of genes vital to the system of immune recognition and reaction. Studies are proposed to determine the structural basis of the mutations in the K or D genes of the H-2 MHC which are responsible for histogenic and, in some cases, serological reactivity with the parent strains. Analysis of the mutant products will be carried out by tryptic peptide mapping to determine if differences exist. Localization of tryptic peptide differences will be made to specific CNBr fragments in order to localize the mutational site within the molecules. Sequence analysis from selected mutants will determine the nature of the molecular alteration of the mutation.

84. F1 ANTIPARENTAL CELL-MEDIATED LYMPHOLYSIS IN VITRO

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Mouse F1 spleen cells develop cytotoxic activity in vitro against parental cells. The antigenic differences detected appear to be controlled by a structural gene (Hh-1) mapping in or near the D region of the H-2 complex. Although a number of positive correlations have been demonstrated between murine hemopoietic graft rejection in vivo and F1 anti-parent cytotoxicity in vitro, certain differences have been found between these two F1 anti-parent reactions. These include the observations that marrow graft rejection does not require priming and is not dependent upon thymus-dependent cells, whereas the development of cytotoxic cells requires sensitization and is effected by T-lymphocytes. The same mechanism is also responsible for the rejection of hemopoietic allografts. Injection of F1 mice with parental spleen cells (which have graft vs. host potential) results in the specific abrogation of F1 anti-parent reactions in vitro and in vivo, whereas injection of parental bone marrow is associated with some degree of priming for F1 anti-parent cytotoxicity.

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85. EVALUATION OF GENETIC RESTRICTION IN THE ACTIVATION OF HELPER T-CELL FUNCTION

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Our studies to date reveal that spleen cells from athymic (BALB/C (H-2d)) nude mice are capable of generating a primary in vitro antibody response to SRBC when provided with either H-2 syngeneic or allogeneic gamma-irradiated (2000 rad) or mitomycin c-treated T-cells from spleens or lymph nodes. However, a similar helper function, if provided by thymocytes, is strictly H-2 restricted. The experiments proposed in this project are primarily aimed at determining the nature of this apparent disparity in the expression of H-2 linked helper function between T-cells obtained from lymph nodes/spleens and those obtained from thymocytes. The precise questions posed towards this goal are: (a) Which H-2 loci are involved in the H-2 linked 'help'; (b) possible role of macrophages in the expression of H-2 restricted help; (c) population of thymocytes that exhibit the restriction; (d) form in which 'help' is made available (e.g. cell-to-cell contact or soluble mediators) both in restricted and in

unrestricted helper function; and (e) use of metabolic inhibitors in conjuction with gamma-irradiation to evaluate early molecular events leading to the generation of helper signal. Understanding the nature of helper signal will eventually facilitate the generation of CTLs directed against the CD determinants of sygeneic tumor cells.

86. REGULATION OF THE MHC RESTRICTED CYTOTOXIC T CELLS

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MHC (major histocompatibility complex) restricted cytotoxic T cells have an important role in the immune defense against syngeneic tumor cells and also in virus infections. The genetic regulation of this type of T cells has been previously our main field of interest (Hurme et al., J. Exp. Med. 147, 758, 1978, and J. Exp. Med. 147, 768, 1978). Now we are studying two aspects of the regulation of this type of T cell response: first, the hormonal regulation and secondly, the formation of the MHC restriction specificity. In these projects we are using the same models and tissue culture techniques as in our earlier works.

87. FUNCTIONAL INTERACTIONS OF I AND H-2K/D IR GENES

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Genes mapping the H-2K/D and I regions of the major histocompatibility complex (H-2) of mice play a pivotal role in regulating the interactions of lymphocytes in the immune response. The linkage of H-2K/D and I region analogs has been maintained throughout mammalian evolution suggesting that H-2K/D and I region genes interact to regulate immune responses which are selectively advantageous. I have observed that the T cell response to single non-H-2 histocompatibility (H) alloantigens is regulated by antigen-specific immune response (Ir) genes mapping in both I and H-2K/D regions. I propose to conduct a comprehensive genetic and immunological analysis of the functional interactions of these two Ir gene systems. The Ir genes which control recipient responsiveness in vivo and in vitro will be characterized by determining their H antigen specificity, minimum number, and intra-H-2 map positions. Similarly, the Ir genes which regulate the presentation or immunogenicity of non-H-2 H antigens will be mapped and their H antigen-specificity determined. The possible preferential association of single H antigen-specific fast responsiveness and high immunogenicity alleles at I and H-2K/D loci in specific H-2 haplotypes will be investigated; this investigation is aimed at revealing I:H-2K/D linkage disequilibrium in mice. The cellular level of expression of the genes controlling recipient responsiveness will be determined by Ly phenotyping T cells responding to non-H-2 H antigens. Similarly the identity of cells presenting non-H-2 H antigens will be determined. The suspected role of I and H-2K/D Ir genes in antigen-specific T cells and H antigen-positive stimulator and target cells will be confirmed. Techniques will be employed to demonstrate that non-H-2 H antigens associate with Ir gene products, which have variable affinity for H antigens, to form immunogenic complexes on the cell membrane. Complementary experiments will be conducted to determine the specificity of the T cell receptor(s) for non-H-2 antigen and 'self' H-2 complex molecules by serological characterization of the respective T cell receptor idiotypes.

88. GENETIC ANALYSIS OF NATURAL KILLER CELLS AND HYBRID RESISTANCE IN MICE

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OBJECTIVE: The objective of this study is to further analyze the H-2 linked genes which are involved in the regulation of natural killer ('NK') cell activity and hybrid resistance against tumors in mice, and to critically assess the linkage between these two phenomena.

BACKGROUND: Murine NK-cells can lyse certain tumors of syngeneic, allogeneic and xenogeneic origin in vitro, but the basis for their selectivity and the nature of the target structures they recognize have not been clarified. The killer cells are thought to be lymphocytes, but they lack conventional B- and T-cell markers. Inbred mouse strains display different lytic efficiency in the NK-assay, and such high efficiency or 'NK-activity' is inherited dominantly in F1 hybrid crosses. Segregation analyses have indicated polygenic control with at least one H-2 linked gene. The resistance of certain F1 hybrid and backcross mice against growth of small inocular or NK-sensitive tumors correlates well with NK-activity of the same strain combination and is also H-2 linked.

PLANS: Crosses between normal inbred mouse strains and congenic or recombinant strains, which differ only at the H-2 or a small part of H-2, are being used for in vitro NK-tests as well as in vivo resistance tests against various tumors. These tumors differ with regard to inducing agent and histologic origin, and we aim at analyzing whether the same genes are involved in regulation of 'general tumor resistance', or whether there exist genes which regulate resistance only in certain strain combinations and/or against certain tumors. The latter possibility would imply different effector mechanisms in the rejection of certain tumors, and NK-cells may be responsible for some of these. Their role is further analyzed by testing the growth of tumors in the mutant strain B6, beige, which completely lacks NK-cells, but has other immune functions intact. The role of T-cells is analyzed with thymectomized mice in certain strain combinations. In order to investigate the genetic regulation and possible role of NK-cells in resistance against primary oncogenesis, some of the congenic, recombinant and mutant strains will also be injected with oncogenic viruses (Rad-LV and Moloney virus) or treated with carcinogenic drugs.

89. GENETICS AND BIOLOGY OF THE HISTOCOMPATIBILITY-LINKED COMPLEMENT GENES AND THEIR RELATIONSHIP TO TUMOR RESISTANCE

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The objective of the project is to analyze the genetics, polymorphism and function of the complement genes linked to the major histocompatibility gene complex. These genes control the level of the components C4, C2 and BF. These components are partly under hormonal control. Their role in the control of immune reactivity and their activation by viral particles and by cell surfaces of transformed and normal cells will also be studied.

90. H-2 ASSOCIATED NATURAL RESISTANCE

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Natural resistance in vivo is studied by injecting unimmunized mice with tumor cells prelabelled with the thymidine analogue 131 I-iododeoxyuridine. A characteristic of all mice tested was the ability to recognize and kill leukemia cells which were not identical with self at the major histocompatibility complex (H-2). By monitoring the survival of the prelabelled cells with whole body gamma counting and determining their metastatic distribution, we have found that resistance is manifest as a decrease in survival of the total tumor cell inoculum, and as a failure of the cells to survive in the spleens of mice which are not H-2 identical with the tumor. This resistance is not due to classical immune responses. In some strains of mice resistance is abolished by whole-body irradiation while in others resistance is left intact. Resistance by irradiated mice of the C57BL family depends on H-2D (Hh-1) nonidentity between the host and the graft. The more extensively studied phenomena of haemopoietic graft rejection by irradiated mice and in vitro natural killer activity may be subsets of a more general para-immune resistance system. We propose to extend our studies on natural resistance through genetic analysis of the recognition process, including the nature of the target 'antigen', and through determination of the genetic and physiological bases which determine the radio-susceptibility of the rejection process. The mechanisms involved in natural resistance will also be studied. Natural resistance is the earliest manifestation of alloreactivity and its relationship to the subsequent development of the immune response will be determined. A major portion of this project is aimed at determining whether natural resistance is a manifestation of a primitive paraimmune surveillance system against aberrant hae-

mopoietic cells. This will involve studies on resistance to H-2 identical Friend and Abelson leukemia virus induced tumors. In addition to its possible significance in resistance against neoplasia, the study of natural resistance could provide valuable information pertinent to the evolution of adaptive immunity, the control of haemopoiesis and cell interactions, and the physiological functions of the major histocompatibility complex.

91. MAJOR HISTOCOMPATIBILITY COMPLEX IN THE WILD RAT

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This research proposal is concerned with comparative studies of the major histocompatibility complex (MHC) in inbred and wild Norway rat populations. We have previously demonstrated that the genetic loci in the RT1 complex that control the expression of serologically defined histocompatibility and Ia antigens, the mixed lymphocyte response, and the immune response to synthetic polypeptide antigens are much less polymorphic than expected. In addition, these different loci in random populations exhibit high degrees of linkage disequilibrium, suggesting that selective pressures are preferentially influencing the retention of selected MHC haplotypes.

The major emphasis of the experimental work of this grant application will focus on three areas. The first is a continued examination of the genetic fine structure of the MHC, particularly with regard to identifying and isolating new wild haplotypes that may allow identification of new loci within the complex. The second is the investigation of wild MHC haplotypes with genetic variation in the RT1.B region in an attempt to establish the genetic and functional relationships between the loci that code for the expression of Ia antigens, the mixed lymphocyte response and the immune response to simple antigens. The third area of investigation will be to establish the genetic control and expression of cell surface antigens that function as targets for the cell-mediated lympholysis reaction. Once identified, the influence of these antigens on the success or failure of kidney allografts exchanged between inbred and wild lines will be examined.

The ability to detect specific components of the MHC in individual wild rats provides the opportunity to compare, simultaneously, inbred and wild populations of rats, allowing for the generation of natural genetic recombinations within the MHC and examination of how this important complex behaves under natural conditions.

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92. CELLULAR ANTIGENS OF NORMAL AND MALIGNANT RAT TISSUES

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Histocompatibility genes, mapping to the major histocompatibility complex (MHC), code for cell membrane components which play a prominent role in the regulation of the immune response. MHC genes regulate lymphocyte coilaboration, antigen presentation to effector lymphocytes, immune responsiveness, and susceptibility to neoplastic and immune diseases.

The vast majority of information on the immunological function of the MHC has been obtained in the mouse MHC (H-2). Analyses of mouse and human MHC's have suggested that MHC's in different species may possess unique characteristics despite unifying similarities. Therefore, caution should be exercised in offering generalities about MHC organization and function based on observatons from a limited number of species.

The major efforts of this research are concerned with the comprehensive analysis of the rat MHC, RT1. Emphasis will be placed on the following studies: (1) serological and biochemical characterization of rat Class II (Ia) antigens with the intention of elucidating the extent of polymorphism at the two Class II loci in the rat, (2) identification of intra-RT1 recombinants in order to delineate the RT1 linkage map, and (3) identification of previously undiscovered RT1 genes. The proposed studies will have a major impact on the knowledge of the genetic organization of rat MHC genes, the molecular characteristics of their products, and their immunological function.

93. THE BIOLOGICAL BASIS FOR HETEROGENEITY OF YEAST HISTONE GENES

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This research proposal is directed toward determining the biological basis for the heterogeneity of the yeast histone genes. The yeast Saccharomyces cerevisiae contains histones H2A, H2b, H3, and H4 very similar to those found in higher eukaryotes, although the existence of a protein analogous to histone H1 has not been convincingly demonstrated. However, unlike higher eukaryotes, the haploid yeast genome has only two copies of the histone H2A gene and two copies of the histone H2B gene. This feature plus the recent development of efficient techniques for transformation of yeast will allow me to determine if both gene copies of yeast are functionally equivalent.

Specifically, I will first determine when each H2B gene copy is transcriptionally active by identifying the specific H2B mRNA present in vegetative and sporulating cells. Then by replacing a wild type H2B gene with a nonfunctional H2B gene, I will be able to determine why there are two histone H2B genes in yeast. Perhaps there is a differential expression of the two gene copies during the developmental cycle of yeast-mitotic growth and meiotic growth (sporulation). Indeed, expression of major histone-like proteins specific to meiosis has been reported in many organisms from echinoderms to mammals.

B. BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF HISTOCOMPATIBILITY ANTIGENS

1. CHARACTERIZATION OF HLA ANTIGENS

94. HLA-DR-LIKE ANTIGENS ON HUMAN MILK FAT GLOBULE MEMBRANE AND BREAST EPITHELIUM

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The milk fat globule membrane (MFGM) originates from the mammary epithelium during lactation by enveloping globules of fat during the secretory process. Suspensions of MFGs were positive when tested with a rabbit anti-DR serum or mouse hybridoma monoclonal anti-DR antibodies (DA2).

Examination of formalin-fixed preparations of breast tissue showed DR antigens to be localized on the secretory epithelium, although these were found only on lactating breast sections. Non-lactating breast, carcinomas or benign tumors, cultures of normal breast epithelium (R.C. Hallowes), a pleural effusion line MCF-7 or epithelial cells from milk (S. Chang) were negative.

Antisera raised against MFGM after absorption with epithelial membrane antigen (EMA) possessed residual anti-DR activity as judged by their ability to bind to DR-positive lymphoid cells or to precipitate two bands of c. 34,000 and 23,000 daltons from radio-labelled lymphoid DR-positive cell lines.

Further investigations will establish whether DR antigens from hemopoietic origin differ from those of epithelial origin and it is hoped that a large scale purification of DR antigens from MFGM will also be possible.

95. CHARACTERIZATION OF THE PHOSPHORYLATION SITE OF THE HLA-DR ANTIGENS

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The overall aim of this research is to characterize the phosphorylation site of the HLA-DR antigens, the human histocompatibility antigens located at the surface of cells of the

immune system. This investigation will examine the role of phosphorylation in the regulation of movement of the DR antigens in the plasma membrane. The mechanism of regulation involves binding of the antigen to the cytoskeletal elements composed of actin. This movement has previously been shown to be associated with immune functions involving recognition and induction of proliferation.

The proposed research involves chemical cleavage of the DR polypeptide and isolation of the phosphate containing fragments which will then be sequenced. The actin binding properties of the HLA DR will be studied using competetive binding studies with myosin, spectroscopic chemical labels, chemical crosslinking and ultracentrifugation. The structural features of the putative actin binding site will then be characterized and the mechanism of its regulation by phosphorylation will be determined.

96. STUDY OF TRANSPLANTATION AND TUMOR ANTIGENS

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We have continued our research leading to identification and characterization of the antigenic factors making up the major histocompatibility locus in man that are important in organ transplantation. We have identified a number of additional B cell specific antigens of the HLA-Dr system and have studied their inheritance in families. We have completed studies on a number of black Americans and have shown that 5 new B cell specificities are identified in this patient population. In contradistinction to Caucasians, black Americans typed for HLA-D with homozygous typing cells failed to exhibit strong linkage disequilibrium between D and Dr types. This could prove to be important in transplantation between these ethnic groups. We have additionally continued our studies concerning the association of tumor associated antigens present on the cell membrane and have now prepared monospecific antibodies to human melanoma antigen and have characterized a human anti-human alloantibody, a chimp anti-human xenoantibody, and a monoclonal antibody prepared by fusion of mouse myeloma cells with specifically sensitized human lymphocytes against melanoma. These antisera specifically localize human tumors in the in vivo setting. We have begun developing methodology for in vitro induction and assay of blastogenesis, cytotoxic lymphocytes, and suppressor cell generation using appropriate tumor cells or PBL from HLA non-identical individuals as targets. Using our mono-reactive anti-tumor antibodies we have completed immunofluorescence and immunoprecipitation studies providing physical-chemical analysis of the antigens present on the surface of human melanoma tumor cells. Cocapping and coprecipitation studies using these reagents are in progress to assess the physical-chemical association of the tumor antigens to the Dr antigens and MHC antigens expressed on normal cells.

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97. HLA RESTRICTED SUPPRESSOR T CELLS OF MIXED LYMPHOCYTES

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In the past year, a number of new findings were made possible by the availability of monoclonal antibodies to lymphocyte surface markers. Monoclonal antibody to HLA-DR antigen was used to study the source, distribution and functions of HLA-DR molecules on T and non-T lymphoid cells. The results indicate that DR is required for stimulation in autologous mixed lymphocyte reaction (MLR) and presentation of soluble antigen. Resting T cells do not synthesize or express detectable DR molecules but alloactivated T cells (purified with a pan-T monoclonal antibody) synthesize and express significant amounts of DR that is identical in charge and molecular weight to B cell DR from the responder. Most importantly, DR positive but not DR negative alloactivated T cells were suppressive of fresh MLRs and suppression was specific for the DR molecules of the stimulator cells; such antigen-specific suppressor T cells were inducible in all healthy individuals tested. In other studies, genetically restricted MLR suppressor T cells of a healthy donor were isolated from peripheral blood with a monoclonal antibody that recognized only 20% of the donor's T cells. All MLR suppression was mediated by these cells and these cells elaborated antigen-specific soluble suppressor factors. In another study, we showed that total lymphoid irradiation (TLI), a standard treatment for Hodgkin's disease and an experimental approach for the treatment of autoimmune disorders and prevention of allograft rejection, induced circulating MLR suppressor cells in patients who lack such cells prior to treatment. TLI-induced suppressor cells were nonspecific with respect to antigen and not genetically restricted with respect to responder. Thus, 3 types of MLR suppressor cells have been observed: 1) Genetically restricted cells of rare healthy individuals; 2) Antigen-specific, genetically unrestricted alloactivated cells; 3) Antigen nonspecific genetically unrestricted suppressor cells induced in vivo by TLI.

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98. LYMPHOCYTE CELL SURFACE ANTIGENS, NORMAL AND NEOPLASTIC

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The biologic, functional and chemical characterization of human lymphoid cell surface antigens controlled by genes of the major histocompatibility complex (MHC) is under investigation. HLA typing of disease groups and families wherein several members are diseased has demonstrated a complex association of the MHC with the disease condition. Antigens not found in linkage disequilibrium in a normal population are found to mark psoriasis and psoriatic arthritis. Connective tissue diseases with overlapping clinical manifestations have distinct distribution of HLA alloantigens suggesting specific genetic contributions of the MHC to the disease state. Alloantisera detecting murine Ia antigens cross-react with human B lymphocyte and monocyte antigens (HLA-Dr). Human alloantisera detecting HLA-DRw antigens precipitate combinations of molecules of 4 different molecular weights. These antisera, when reacted with monocytes, induce specific T cell suppression of immunoglobulin synthesis and antigen stimulation. Monoclonal antibodies to lymphocyte cell surface antigens appear to identify functional subsets of T lymphocytes.

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99. IDENTIFICATION OF HLA-D/DR DETERMINANTS

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OBJECTIVE: To identify and further characterize the HLA-D/DR determinants, on normal cells and malignant cells.

APPROACH: The DR determinants are identified by serology and the D determinants by cellular methods (HCT, PLT). The antisera are mainly produced by planned immunization.

PROGRESS: Our own serological reagents have been shown to identify DR antigens, and we have also demonstrated that it is possible by serology to type for the D determinants. Their further tissue distribution on normal and malignant cells is under study.

100. HUMAN HLA-D ANTIGENS

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A gene product from the human histocompatibility locus, HLA-D, has recently been purified from a B lymphoblastoid cell line. Initial studies of the dimeric structure showed features closely analogous to those of the polymorphic murine and guinea pig 'Ia' antigens found on the surface of several types of immunocompetent cells. Furthermore, like the murine Ia antigens, this human HLA-D antigen appears to play a role(s) in the regulation of the immune response. The structural basis for theoretical regulatory functions is not known.

Using specific heteroantisera against three different purified HLA-D antigens (which should have some overlapping but different specificities), I propose to locate and isolate human HLA-D antigens from several different cell types (T cells, B cells, macrophages and leukemic cell lines). Then I will comparatively examine their size and subunit structures and thus further determine the degree of analogy to the mouse and guinea pig systems. Following immunoprecipitation, initial structural characterization will be obtained using SDS-gel electrophoresis. After partial purification by affinity chromatography, peptide mapping will help establish the degree of homology between various subunits. Eventually, purification of HLA-D antigens from cell types other than B cells will allow more detailed immunochemical and biochemical analysis and provide an answer to the important question of whether HLA-D antigens on different cell types are products of the same gene or of closely linked genes.

101. STRUCTURAL STUDIES OF HLA DETERMINANTS

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The specific aims of this project are to isolate the polymorphic human histocompatibility antigens (the products of the HLA-A, -B, -C and -D/DR genes in the major histocompatibility complex (MHC) and to elucidate their structures. The molecules which will be studied include HLA-A2, -A28, -B27, -B40, -Cw1, -Cw3, -DRw6 and -DRw8. One of these (HLA-B27) has a very strong association with a human disease. Of special importance is the nature of the alloantigenic site(s) in these molecules which are recognized by alloantibodies and various immune cells such as allo or modified self directed cytolytic T cells. The MHC antigens also mediate various other cell-cell interactions in the immune system. The isolated molecules as well as the structural information about them will be employed in studies of the functions of the molecules. In particular, we wish to explore in as much detail as possible the cytolytic T-cell response both to HLA-A, -B and -C antigens and to HLA-DR antigens and to these antigens modified by chemical means or by interaction with viral antigens. Finally, we hope to use the structural information acquired about these molecules to learn more about how membrane proteins are organized and integrated with cell function.

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102. IMMUNOGENETICS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

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The overall goals of this program are the elucidation of the biological properties of the major histocompatiblity complex (HLA); its role in immunoregulation and relation to disease susceptibility. Identification and characterization of histocompatibility determinants coded for by the known loci of HLA (A, B, C, and D) and identification of other alloantigens and genetic traits coded for by the HLA-linkage group will increase the quality of histocompatibility testing and subsequently result in improvement in clinical transplantations. This program focuses on the study of histocompatibility determinants in normal individuals and in different patient populations. The individual projects of the program emphasize: 1. The complexity of the HLA-D genetic region (which governs the lymphocyte activation in the mixed lymphocyte culture reaction) and the genetic control of alloantigens selectively expressed on B-lymphocytes (DR determinants); 2. analysis of the genetic control of generation of cytotoxic lymphocytes and lymphocytes with specific immunological memory in allogeneic in vitro reactions; 3. definition of the distribution of restricted alloantigens on lymphoid subpopulations and the study of the functional characteristics of these lymphocytes in order to identify their role in immunoregulation; and 4. the study of associations between histocompatibility determinants and diseases.

103. CELL-MEDIATED IMMUNITY TO INFLUENZA-INFECTED AUTOLOGOUS LYMPHOCYTES IN MAN

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Conditions have been developed for: (a) in vitro stimulation of influenza virus-immune cytotoxic effector cells in human peripheral blood leukocyte (PBL) populations; and (b) measurement of effector cell activity on virus-infected cryopreserved human PBL target cells. Effector cells generated in vitro are influenza type (but not subtype) specific and are HLA-A and HLA-B restricted. The cytotoxic response appears to be controlled by HLA-linked immune response (Ir) genes since: (a) cytotoxic activity is predominantly associated with antigens of only one haplotype; and (b) effector cells from certain donors recognize virus in association with some but not all self HLA-A and -B antigens. This Ir gene control is HLA-linked and antigen-specific, since preferential recognition among HLA-identical siblings is different for influenza A and B viruses.

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104. ANALYSIS OF THE HUMAN IMMUNE SYSTEM USING MONOCLONAL ANTIBODIES

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Understanding normal functions of the immune system is critical to understanding disorders such as cancer and immune deficiencies. The molecular products of the human histocompatibility system, the HLA-A, B, C and DRw glycoproteins have been shown to play an important role in cellular immunity. One aspect of this investigation is concerned with examining the structural and antigenic nature of these molecules using monoclonal antibodies directed against them. Another aspect is to produce human monoclonal antibodies with the aim of understanding human humoral immune response and providing medically useful reagents. Three research projects will be pursued. 1) Antibodies recognizing both polymorphic and monomorphic determinants on the HLA-A2 molecule will be used to construct a map of the antigenic sites on this molecule. This will involve making Fab' fragments of the antibodies to use in competitive binding studies to determine the number of distinct antigenic sites. In addition, limited proteolysis of the antigen will be undertaken to find fragments which retain antigenic activity in order to correlate antigenic sites with known biochemical features. This analysis may explain the efficacy of certain antibodies in inhibiting HLA-dependent cellular processes and demonstrate which regions of the molecule are involved in these processes. 2) Comparison with the mouse histocompatibility system suggests that it should be possible to find additional

antigenic products from HLA loci similar in molecular weight to the HLA-DRw antigens. Preliminary evidence from antibody binding studies suggests that one monoclonal antibody reacting with the HLA-DRw antigens reacts with this other set of antigens as well. These antibodies will be used to purify this second population of antigens which will then be characterized in terms of molecular weight and tissue distribution and compared to similar mouse histocompatibility antigens. Identification of human equivalents of some of the characterized mouse antigens may suggest a possible function for these molecules in the human system. 3) The monoclonal antibody production technique will be adapated to the human system by searching for human myelomas which successfully fuse and confer antibody production and by developing methods for in vitro antibody production.

105. A STUDY TO DEFINE A NEW LEUKOCYTE HISTOCOMPATIBILITY SYSTEM IN MAN

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OBJECTIVE: To define B lymphocyte alloantigens in man and to establish their relevance to lymphoproliferative disorders.

APPROACH: Sera from ante-natal, multitransfused, transplanted and leukemic patients are screened against cells from patients with CLL and other B cell proliferative diseases and their patterns of reactivity analyzed with reference both to the newly defined DR antigens and also to the particular diseased cells being used in the hopes that some sera may prove useful diagnostically.

PROGRESS: This work has produced many antibodies which can be used for typing B cell antigens and some of these were used in the 7th Histocompatibility Testing Workshops, Oxford 1977.

106. IDENTIFICATION OF DIFFERENTIATION ANTIGENS ON HUMAN LYMPHOCYTES

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We propose to prepare a series of hybrid cell lines that produce monoclonal antibodies to human lymphocytes. Our primary goal is to obtain monoclonal antibodies specific for T cells. Fusion experiments will be performed with mouse spleen cells immune to normal T cells, activated T cells, thymocytes, and cultured leukemic cell lines. Our strategy for selecting specific hybrid clones will be to utilize pairs of matched or autologus normal or activated lymphoid cells, or leukemic lines. These reagents will be used to identify functionally distinct subpopulations of T cells, especially cytotoxic T lymphocytes and their precursors, helper cells and suppressor cells. Monoclonal antibodies obtained will be used to study differentiation antigens as they are expressed by cultured hematopoietic cell lines, cultured leukemic lines and leukemic blasts. Clinically, we will use these reagents to characterize the alterations occurring in the lymphocyte subpopulations of patients with acute and chronic GVH disease. One of the central objectives will be to explore the feasibility of using monoclonal T cell antibodies for the prophylaxis or treatment of GVH disease.

107. HUMAN TUMOR ANTIGENS - RELATIONSHIP OF TUMOR ANTIGENS AND TRANSPLANTATION ANTIGENS

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The rejection of tumor grafts between animals suggests that tumor antigens operationally can behave as transplantation antigens. This, coupled with other recent evidence, suggests that tumor antigens may in fact represent a modified histocompatibility antigens. Our objective has been to demonstrate a possible structural similarity between tumor and histocompatibility antigens. It was hypothesized that if such a relationship exists, beta-2-microglobulin, the species-specific low molecular weight chain common to the histocompatibility antigen of all species, would be similarly expressed in the putative tumor antigen. Affinity chromatography employing anti-human beta-2-microglobulin antiserum was used to purify both the HLA and putative HLA-like tumor antigen from normal and cancerous tissues. Using an in vitro assay for tumor antigen activity (leukocyte adherence inhibition, LAI), we found that material purified by this affinity chromatography method possessed tumor antigen activity. The material so purified demonstrated an HLA-like subunit structure on DSD-polyacrylamide gels. Presently, antitumor antibody is being prepared using the monoclonal antibody method.

108. IMMUNORESPONSE TO HUMAN SURFACE ANTIGENS

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Mouse-human somatic cell hybrids containing either human chromosome 6, or 7, or 16, or 17, or 17 carrying the region p21 yields pter of human chromosome 6 as the only human chromosome present in the hybrids have already been produced and injected into syngeneic mice. Sera from hyperimmunized mice will be used to characterize the surface antigens coded by these human chromosomes and to further localize the position of the genes coding for these antigens on the respective human chromosomes. We will also determine whether the cellular immunoresponse to human histocompatibility antigens expressed in hybrid cells containing human chromosome 6 or part of human chromosome 6 is H2 restricted. The expression of H2 and HLA antigens will also be studied in hybrids between F9 mouse teratocarcinoma cells and either mouse cells derived from different mouse strains or human cells to determine whether in the undifferentiated hybrid cells the expression of H2 and HLA antigens is shut off and whether concordant expressions of mouse and human histocompatibility antigens occur in the differentiated hybrid cells. Syngeneic mice will also be hyperimmunized with somatic cell hybrids containing either chromosome 6 or the region p21 yields pter of human chromosome 6 and expressing HLA 3 and 7 or HLA 32, and their spleen cells will be fused with P3x63Ag8 mouse myeloma cells to obtain hybrids which produce antibodies against human histocompatibility antigens. This study should result in the selection of a series of hybrid clones producing monoclonal antibodies to different human histocompatibility antigens.

In addition, we intend to study the cellular and humoral immunoresponse to Epstein-Barr (EB) virus nuclear antigen (EBNA) and surface antigens using somatic cell hybrids between mouse cells and Burkitt's lymphoma cells.

109. HUMAN IMMUNE RESPONSES TO HAPTENS IN VITRO

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The overall objective of this proposal is to develop a model for studying human immune responses to neoantigens on cell surfaces. To this end we will exploit techniques which we have recently developed for induction of human cell-mediated immune responses in vitro to hapten-conjugated cells. We plan to investigate both primary and secondary responses in a proliferative assay system. Most experiments will utilize trinitrophenyl-conjugated cells. However, dinitrophenyl and fluorescein conjugates will also be studied. The role of major histocompatibility complex genes and gene products in such responses will be of particular interest in two respects. First, we will investigate the possible role of HLA-associated Ir genes as determinants of responsiveness to hapten-conjugated cells. These studies will be performed in families, in an effort to associate high or low responsiveness with the inheritance of the HLA complex. Second, we will characterize the role of HLA-antigens in induction and elicitation of responses to hapten-conjugated cells. Stimulator and responder cell populations involved in these responses will be characterized functionally, physically and for cell surface properties. These studies should provide fundamental insights into T cell recognition and response in humans and the role of major histocompatibility genes and antigens in such responses. By providing a practicable in vitro model for evaluation of human immune responses to cell surface neoantigens, this work will also have important implications for studies of immune responses to neoplastic cells.

110. DEFECTIVE SELF-RECOGNITION AS A CAUSE OF CANCER

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Murine cytotoxic effector cells and their progenitors recognize membrane-bound viral and tumor antigens only in association with H-2 histocompatibility (H) antigens. In man an analogous HLA restriction of cytotoxic responses to trinitrophenol (TNP) and the Y-linked antigen has been described. Immune response genes in the mouse control the ability to recognize self, and there are examples of defective genes. Stains bearing these genes cannot generate cytotoxic responses to H-2 restricted antigens, not because they cannot recognize the antigen but because they cannot recognize self. Some humans may similarly be defective in the ability to recognize some of their own H antigens. It is possible that tumors escape immune survillance if their antigens become associated by selective survival with those H antigens which the host is unable to recognize for genetic reasons. It is proposed to test these hypotheses: some patients with cancer have a defect in their ability to recognize self; their tumor arose partly as a consequence of this defect; the defect is hereditary and linked to HLA. These hypotheses can be tested in families of cancer patients with the use of recently developed methods for in vitro sensitization of human peripheral blood lymphocytes in TNP-modified autologous lymphocytes and for assay of these cytotoxic cells on TNP-modified allogeneic targets with shared H antigens. The identification of such defects and the demonstration of a causal relation between the defect and the appearance of a tumor has important implications for the detection of cancer-prone individuals. Correction of the defect, as through the transplantation of responder lymphocytes, might then be a particularly effective form of specific cancer prophylaxis.

111. URINE AS A SOURCE OF HUMAN CELL SURFACE MARKERS

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The major objective will be to elucidate the molecular structure of the allotypic sites of human histocompatibility antigens. To this end, highly purified antigens expressing different phenotypes will be selectively cleaved with proteases and antigenically reactive peptides will be isolated by affinity chromatography with monoclonal antibodies. Such peptides will be analyzed for molecular size and amino acid sequence to determine their location within the overall molecular structure of histocompatibility antigens and to delineate the specific molecular structures which are associated with known allotypic specificities of human histocompatibility antigens.

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112. CHEMICAL STRUCTURE OF IMMUNOGLOBULIN MOLECULES, ALLOTYPES AND HUMAN HISTOCOMPATIBILITY ANTIGENS (ABBREV)

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Chemical and Structural Studies of Antibodies, Histocompatibility Antigens, Tumor Antigens and Epoxide Hydrase: It is the purpose of this project to study the chemistry and structure of different molecules. The amino acid sequence of light and heavy chain immunoglobulins is obtained in order to gain some insight into the nature of antibody specificity and the generation of diversity. Biochemical isolation of histocompatibility antigens, tumor antigens and epoxide hydrase is carried out. The chemical structure of these proteins is being approached by micro methods and the relationship of the amino acid sequence to immunoglobulin structure or other known structures is being analyzed. Cloning of histocompatibility antigens is being approached in order to study the molecular organization of the system and permit a better understanding of the nature of the extensive polymorphism at the H-2 loci.

BIBLIOGRAPHIC REFERENCES: Law, L.W., Rogers, M.J., and Appella, E.: Tumor antigens on neoplasms induced by chemical carcinogens and by DNA- and RNA-containing viruses. Properties of the solubilized antigens. In Weinhouse, S., and Klein, G. (eds.): Advances in Cancer Research, New York, Academic Press, 1980, Vol. 32, pp. 201-235. Appella, E.: Amino acid sequence of the light chain variable region of M511, a phosphorylcholine-binding murine myeloma protein. Molecular Immunology, in press, 1980.

2. CHARACTERIZATION OF H-2 ANTIGENS

113. GENETIC REGULATION OF THE EXPRESSION OF HISTOCOMPATIBILITY ANTIGENS ON TUMOR CELLS

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DESCRIPTION: Histocompatibility antigens seem to play an important function in immune surveillance of virus-infected and malignant cells. Recently we have demonstrated differences in the expression of individual histocompatibility determinants on tumor cells as compared to normal cells. In this project, we aim to further characterize the new H-2-like specificities which were observed. Furthermore, we aim to investigate the genetic control of the expression of histocompatibility antigens on tumor cells, particularly potential repression and derepression systems.

114. ADJUVANT EFFECT OF ALLOGENEIC HISTOCOMPATIBILITY ANTIGENS IN IMMUNOLOGICAL REACTION AGAINST TUMOR-ASSOCIATED ANTIGENS

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OBJECTIVE: To test, for therapeutic purposes, the possibility of increasing the host response to weak tumor-associated antigens by means of allogeneic histocompatibility antigens carried by the same cell.

APPROACH: Our previous finding on rat skin homograft survival (Experientia, 30, 947-949, 1974) and very recent data (Fed. Proc., 37, 2052-2054, 1978) on hamsters have shown a clear adjuvant effect. We aim to detect such a phenomenon in in vivo and in vitro systems using allogeneic SV40-, M-MSV-, and RSV-transformed cells, as well as mouse lymphosarcoma and embryo cells as sensitizers. The parameters studied are: tumor growth, host survival and in vitro cytotoxicity of spleen cells.

PROGRESS: In vitro cytotoxicity (3H-proline test) against syngeneic SV40-transformed (KB/cSV) cells was evaluated in BALB/c mice after immunization with either KB/cSV and KD2SV cells. (The latter, obtained from SV40-transformed cells of B10.D2 mice, show the same MHC (H-2d) while presenting a different pattern of minor histocompatibility antigens.) A higher cytotoxicity was not observed when immunizing with KD2SV cells. Thus, an adjuvant effect by minor histocompatibility antigens in reaction against antigens associated with the transformation by SV40 has not been evidenced in this experimental design. After immunization with KB/cSV cells, a cytotoxicity higher than that observed using KD2SV cells as immunogen was frequently found; the possible relationship between this finding and age of the animal is under investigation.

115. IMMUNOLOGICAL AND GENETIC CONTROL OF EXPERIMENTAL ONCOGENESIS IN MICE (ROLE OF DIFFERENT REGIONS OF MHC)

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The objectives of this project are a) to evaluate the role of the different major histocompatibility complex (MHC) regions in the immunological response to RNA virus-induced tumors of mice, and b) to determine the presence of possible modifications of antigens coded by the MHC in spontaneous and virus-induced tumors of mice.

The experimental model employed consists of tumors induced by Moloney sarcoma virus (M-MSV) or by Moloney leukemia virus (M-MuLV) in different strains of mice, including several H-2 recombinant strains. The immunological response of lymphoid cells from M-MSV injected mice is evaluated by means of a 51Cr release assay directly (I-CRT) or following in vitro stimulation with antigenically related tumor cells (2-CRT). Target cells consist of leukemic cell lines derived from primary lymphomas originally induced in different H-2 recombinant muse strains by neonatal infection with M-MuLV. The presence of possible H-2-like alien specificities on tumor cells is determined by serological methods and by T cell-mediated cytotoxicity. Spontaneous tumors of SJL (H-2s) mice or M-MuLV-induced tumors from other strains sharing all (ASW) or only part (ATH; ATL; BIO.HTT) of the H-2s haplotype are studied. Inappropriate specificities have been described recently with a high frequency of occurrence both on normal as well as on tumor cells from SJL mice.

116. H-2 LINKED RESISTANCE TO TUMOR - EFFECTORS AND TARGETS

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The ultimate objectives of this proposal are to determine the role of natural resistance and the functions of the host's genetic composition in host defense against tumors. The immediate objectives of this proposal are to establish the in vivo and in vitro models for natural host resistance against murine tumors which is determined by genes linked to the host's major histocompatibility complex and to define the effectors and the nature of target antigens involved in the reactions. In the in vivo model, histocompatible F1 hybrids of DBA/2 mice with various congenic H-2 recombinant strains will be used as hosts to test their resistance against the growth of the DBA/2 lymphoma L1210 and three drug-resistant L1210 sublines. These sublines have previously been shown to have a high density of tumor-associated antigens (TAA). We will study whether the resistance of the F1 hybrids against the sublines differs from that of the L1210 and determine the effects of the host's genetic composition coded by the H-2 as well as non-H-2 loci. In the in vitro model, lymphocytes of normal histocompatible F1 hybrids will be cultured with irradiated cells of these tumors. The antibody-forming cell (AFC) and cell-mediated cytotoxic (CMC) responses will be measured by the plaque assay and the 51Cr-release cytotoxicity test, respectively, using the tumor cells as target. The in vitro results will be compared with the in vivo results. Effectors and their functions in the in vitro responses will be thoroughly characterized: cell types will be determined by the tests for surface characteristics of B cells, T cells, NK cells and macrophages, and requirements for accessory cell functions will be examined by selective depletion and reconstitution of cell preparations with the functional cell subpopulation. Hybridoma-produced monospecific antibodies directed to TAA or other relevant surface antigens on tumor cells will be used in the antibody-mediated specific suppression in attempts to identify target antigens for the in vitro responses.

117. TUMOR-SPECIFIC IMMUNITY AND HISTOCOMPATIBILITY COMPLEX

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The proposed studies are aimed at defining the precise role of products coded for by genes associated with the major murine histocompatibility complex (H-2) in immunity to syngeneic tumors. The experimental model for these studies will consist of methyl-cholanthrene-induced fibrosarcomas derived in congenic, congenic-recombinant and congenic-mutant inbred strains of mice as well as in F1 hybrids appropriate for analysis of the role of H-2 subloci in tumor-specific immunity. These tumors have previously been characterized with respect to in vivo growth, lethality, immunogenicity and alloantigenicity in syngeneic and congenic strains; moreover, the relative alloantigen content of many of these tumors has been determined by serological and cellular assays. Analysis of the immune response to syngeneic tumors will include further resolution of the specificity of hyperimmune effector peritoneal exudate cells (HYPEC) from syngeneic hosts. Systematic probation of HYPEC will be performed in order to exclude or include possible participation of, respectively, non-T or T-lymphocytes, in the in vitro tumor-specific cytolytic response. Antisera from tumor-hyperimmunized syngeneic and congenic hosts will be tested for anti-TSTA directed serological activity (i.e., cytotoxic antibody, blocking antibody, antibody-dependent cell-mediated cytotoxic antibody). Assessment of the TSTA-H-2 intramembrane relationship on methylcholanthrene tumors will be accomplished using the short-term in vitro HYPEC-mediated cytotoxicity test alone and in conjunction with tumor-specific and H-2 directed antisera.

118. THE CHEMICAL BASIS OF THE H-2 RESTRICTION ON CYTOTOXIC T CELLS

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Recent reports indicate that the mouse H-2 complex plays a crucial role in cell-mediated cytolysis (CMC) directed against non-H-2 antigens. These reports demonstrate that histocompatibility between sensitizing and target cells at a portion of the H-2 complex is required for CMC in systems where the non-H-2 antigen is a viral, a minor histocompatibility, or a trinitrophenylated antigen. It has been suggested that this H-2 restriction mechanism may be relevant to in vivo CMC directed against neoplastic cells bearing a tumor associated antigen. To explain this histocompatibility restriction, the favored hypothesis proposed in these reports is that the cytotoxic T cell recognizes 'altered-self'. The object of this proposal is to identify the chemical and/or structural basis of this alteration of self initially in the Sindbis virus system, and subsequently in a minor histocompatibility antigen system. A newly devised radioimmunoassay and cross-linking reagents have permitted the detection of an H-2 antigen-Sindbis antigen association. The nature of this association will be further explored with highly specific antisera to determine if H-2D and/or H-2K antigens are associated with the viral E1 and/or E2 antigens. These associations will also be confirmed by SDS-polyacrylamide gel electrophoresis and isoelectric focusing (IEF). Possible structural modifications of the H-2 antigen by Sindbis antigen (or vice versa) will be analyzed by comparison of IEF patterns, tryptic peptides and carbohydrate moieties derived from 'modified' and 'unmodified' antigens.

119. HISTAMINE H2 RECEPTOR T-SUPPRESSOR CELLS IN CANCER

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Suppressor T-cells play an important role in the biology of human tumors. The focus of the work we propose is the suppressor T-cell subset which is characterized by the possession of a surface receptor for histamine of the H2 type (H2R-positive). Previously we have studied this cell from biochemical and immunological perspectives. This has included the solubilization, characterization and purification of specific H1 an H2 receptors from its surface. In addition, we have demonstrated that the immature percursor of this cell requires thymic stimulation to express the H2 receptor, and have partially purified a factor from calf thymus which specifically induces the H2 receptor. Furthermore, we have shown that this cell can be activated by histamine to become a functional suppressor cell. This effect of histamine is mediated through the H2 site as evidenced by the ability of cimetidine (an H2 antagonist) to block it. We now propose a detailed study of the relationship of this specific suppressor cell to malignant disease. In order to do so we will characterize and quantitate histamine receptors on the T-cells of patients and animals with malignant tumors; determine the effect of histamine and its antagonists/agonists on the functional immunological activity of the H2R-positive cell including lympho-

cyte-mediated tumor cytotoxicity and suppression; determine if histamine and its antagonists/agonists play a role in the therapy of cancer based on studies of their effect on suppressor cells in an animal model; and determine the role that serum histamine may have in the pathophysiology of malignancies. We also will study the plasma and serum of patients with malignancies for the presence of a circulating factor, analogous to that which we have isolated from calf thymus, which induces histamine H2 receptors on precursor T-cells.

120. IMMUNITY TO MAJOR HISTOCOMPATIBILITY ANTIGENS - ANTIBODY AND CELLULAR RESPONSES

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T cells directed at anti-H-2I (A.TH anti-A.TL, 6R anti-AQR) able to help anti-H-2K/D serological responses can be generated in vitro as well as in vivo. We are generating T helper cells with these activities.

A subset of cells directly stimulates primary B cell response to the major histocompatibility complex (H-2 antigens). Neither killed cells nor tumor cells carrying H-2 antigens are immunogenic. The subset thus functions as specialized antigen-presenting cells. The immunogenic cells are: (a) a small subset of cells in spleen and marrow and also, but much more rarely, in lymph node and peritoneal cavity; (b) absent at birth but mature within the first week; (c) unusually dense, radio-resistant and non-adherent; and (d) unable to stimulate in mixed lymphocyte reaction.

Secondary IgM responses to H-2 antigens are obtained in primed mice but are abolished if the priming dose of live cells is large. Killed large cells do not inhibit such secondary responses. Mixed studies indicate that suppressive mechanisms may be involved.

121. NEOPLASIA AND HISTOCOMPATIBILITY ANTIGENS IN SERUM

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One major objective is the use of monoclonal antibodies to assess the structural relationship between histocompatibility and tumor associated antigens on murine tumor cells and to examine possible H-2 viral interactions. Efforts will also be made to further chracterize the immunological and molecular profiles of H-2 and Ia antigens with cross reactive anti-HLA antibodies. The other major objective of our studies is to continue our ongoing efforts to delineate the cellular and molecular heterogeneity of H-2 and Ia antigen on murine cells with monoclonal antibodies.

BIBLIOGRAPHIC REFERENCES: Callahan, G.N., Pellegrino, M.A., Ferrone, S., Haubrich, L. and Reisfeld, R.A. Expression of a sheep red blood receptor by a murine lymphoma. J. Immunol. 123:2725-2728, 1979.

122. H-2 LOCUS AND LOCAL TUMORIGENESIS BY METHYLCHOLANTHRENE

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The objectives of the proposed research are to: 1) Study the genetics of the effect of the H-2 locus on relative resistance to local tumorigenesis by the chemical carcinogen 3-methylcholanthrene (MCA). 2) Determine if the mechanism of this effect involves interallelic interactions or (as has been shown for viral leukemogenesis) cell surface interactions between tumor antigens and H-2-alloantigens.

To these ends we are: 1) Studying the induction of local tumors by MCA in normal and T cell deficient mice differing genetically only at the H-2 locus or portions thereof (i.e., recombinant H-2 haplotypes) and their F1 progeny. 2) Establishing lines of H-2 loss variant cells from cloned tumor cells induced by MCA in mice heterozygous only with respect to the H-2 locus. These H-2 loss variants and their parental H-2 heterozygous cells will be used to determine if MCA-induced tumor associated antigens and H-2 alloantigens interact physically on the cell membrane.

The results of these studies will either support or negate the hypothesis that relative resistance to local tumorigenesis by MCA involves T cell recognition of 'altered-H-2' or 'hybrid antigen' resulting from cell surface interaction between tumor and H-2 alloantigen molecules.

123. THE ROLE OF THE H-2L LOCUS ANTIGENS IN T-CELL MEDIATED CYTOTOXICITY

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BALB/c H-2db spleen cells generated a strong primary in vitro CML response against BALB/c (H-2)d wild type spleen cells. The effector cells generated were specific for L antigens. In an allogeneic CML response between two non-mutant strains directed against H-2D region antigens, two clones of cytotoxic effector cells could be identified, one with specificity for D antigens, and the second with specificity directed against L antigens. When syngeneic cytotoxic effector cells were generated against TNP modified H-2D antigens, no clones with specificity for L-TNP modified antigens could be detected. This contrasts with the H-2 restricted murine influenza model, in which influenza virus can be recognized by cytotoxic T cells in association with products of the L locus. Therefore, although products of the L locus are capable of inducing allogeneic cytotoxic effector cells, and serve as self recognition structures for influenza virus, these antigens appear not to be capable of eliciting syngeneic effector cells specific for the TNP hapten.

BIBLIOGRAPHIC REFERENCES: Levy, R. B. and Hansen, T. H.: Functional studies of the products of the H-2L locus. Immunogenetics 20: 7, 1980.

124. ANTIGENS DETERMINED BY THE MURINE MAJOR HISTOCOMPATIBILITY LOCUS

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Studies are being directed toward understanding the major histocompatibility complex, the structure and function of the products of this complex, and manipulations of immune responses to these products. Current studies include: 1) Characterization of major histocompatibility antigens: Congenic resistant strains of mice are developed, maintained, and used in serologic and immunochemical analyses of the MHC products of the mouse. 2) Studies of monoclonal antibodies to H-2 and Ia antigens: Hybridoma cell lines are produced by fusion of immune mouse spleen cells with mouse myeloma cells. The monoclonal anti-H-2 and anti-Ia antibodies produced by these hybridomas are analyzed by serologic and immunochemical means and are used to further characterize the fine structure of the MHC. 3) Characterization of receptor sites for histocompatibility antigens: Anti-idiotypic antisera are being produced against anti-H-2 and anti-Ia hybridoma antibodies, and the effects of these antisera on in vitro and in vivo parameters of histocompatibility are being assessed. 4) Mechanism of tolerance to H-2 and Ia antigens: The humoral and cellular responses of radiation bone marrow chimeras are being examined, and the mechanism for maintenance of tolerance in these animals is being studied.

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125. BIOCHEMICAL CHARACTERIZATION OF ALIEN HISTOCOMPATIBILITY ANTIGENS EXPRESSED ON MURINE FIBROSARCOMA CELLS

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OBJECTIVE: To compare the peptide composition of alien and original H-2 molecules expressed on methylcholanthrene-induced fibrosarcoma cells to the peptide composition of

H-2 molecules expressed on normal cells. The knowledge of the biochemical characteristics of alien H-2 molecules could provide an important information on the genetic mechanism responsible for the expression of inappropriate determinants on tumor cells.

APPROACH: Normal and alien molecules, metabolically labelled by short-term in vitro culture of tumor or normal cells in presence of radioisotopically labelled precursors, will be purified by immunoprecipitation with specific antisera, followed by chromatography on SDS-PAGE. The purified H-2 molecules will be subjected to treatment with proteolitic enzymes. The resulting peptides will be characterized by ion-exchange chromatography. The peptidic maps of alien antigens and of the corresponding determinants on normal cells will be compared with each other to determine similarity in the primary structures of the molecules.

126. H-2 ANTIGEN EXPRESSION IN CULTURED MOUSE LEUKEMIA CELLS

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The aim of the proposed research is to study the regulation of expression of the H-2 antigens. The approach will be to isolate, from heterozygous mouse leukemia cells in culture, variants that have undergone stable heritable alterations in the expression of the H-2 antigens and to use these variants to investigate the normal controls of H-2 antigen expression. Variants will be isolated by two in vitro techniques. One of these, anti-H-2 antibodies in the presence of complement, has been used successfully in the past two years to isolate several interesting variants. More variants will be sought using this method. In addition, H-2 directed cytotoxic T-cells will also be used as selective agents.

Once isolated, the variants will be studied by genetic techniques such as karyotyping and somatic cell hybridization; biochemical procedures such as gel-electrophoresis and, in collaboration with Dr. S.G. Nathenson, peptide mapping, where warranted; and by immunological methods such as radioimmunoassay, serological responses on inoculation into the syngeneic animal, and interactions with T-lymphocytes, immunized against wild type antigens.

The rates of emergence of these variants will be determined by the fluctuation analysis. This experiment will also be used to determine whether the variants preexist the selection, or are in some way induced by the selective agents. The effect of various mutagens on the rates of emergence will be studied.

Recently, much attention has been focused on the role of the H-2 antigens in the immune response to viral antigens. The H-2 variants and the wild type cells form an isogenic system where this role of the H-2 antigens can be studied. The immune responses of syngeneic animals to the Friend virus associated antigens will be investigated. Some preliminary experiments have suggested that certain combinations of H-2 antigens may interfere with or augment the T-cell response to the antigens associated with the Friend virus.

127. MECHANISM OF THE H-2 EFFECT ON VIRAL LEUKEMOGENESIS

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More than one gene within the major histocompatibility complex of the mouse, H-2, has been shown to influence the susceptibility of mice to viral leukemogenesis, and evidence exists for at least three different mechanisms involved in this effect. The first is the induction of tumor-specific cytolytic T cells in response to recognition of an H-2/viral protein molecular complex formed on tumor cell surfaces. The second is the regulation of the capacity of the host to recognize and respond to viral antigens by H-2-linked immune response genes. A possible third is the influence of H-2 type on the cessation of virus production (replication suppression) by virus-induced tumor cells. Studies of each of these possible mechanisms will be carried out using cultured cell lines derived from Friend virus or Gross virus-induced tumors, or both, of mice of the H-2-congenic BALB/c lines.

BIBLIOGRAPHIC REFERENCES: F. Plata, M.M. Goodenow, F. Lilly, 1980. Studies of cloned Friend erythroleukemia tumor cells. Modulation of the tumor-specific cytolytic T lymphocyte response by infectious Friend virus production in vitro. J. Exp. Med. 151: 726-742. F. Lilly, A. Mayer, 1980. Genetic aspects of murine type-C viruses and their hosts in oncogenesis. In: Viral Oncology (G. Klein, ed.), pp. 89-108. Raven Press, New York.

128. LYMPHOCYTE SURFACE GLYCOCONJUGATES

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It is now evident that cell surface glycoconjugates play a central role in mediating the social responses of cells. We propose to determine the structure of the carbohydrate moieties of the murine histocompatibility H-2K and H-2D antigens and relate this to the serotype of these glycoproteins. We further propose to explore the interrelationships that may exist between the processes resulting in the stimulation of mitosis and production of migration inhibition factor (MIF), lymphotoxin, and chemotactic factor by perturbation of lymphocyte surface glycoconjugates.

Employing sensitive and novel serological, immunochemical and biochemical techniques we will perform partial sequence analysis of both membrane bound and purified H-2K and D antigens to assess the possibility of the influence of primary structure variation of the carbohydrate moiety on the physiologic function of the antigens. These studies will also, by comparing the primary structure of the carbohydrate chains and serotype of various H-2 glycoproteins, assess the possibility of a mechanism which controls and/or links the processes responsible for the biosynthesis of the carbohydrate and polypeptide portions of glycoproteins.

Additional studies will be performed to identify molecular species responsible for MIF, LT and ChF activities. Employing a panel of lectins with different lymphocyte receptor specificities, we propose to dissect the individual pathways of lectin induced MIF, LT and ChF by monitoring the appearance of specific molecular species and activities characteristic of these lymphokines.

129. CELL SURFACE ANTIGENS ON MALIGNANT LYMPHOCYTES

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Possible antigen interactions occurring between the tumor-associated surface antigen (TASA) and the K and D regions of the major histocompatibility complex (H-2Kk,Dk) on the surface of malignant line Ib lymphocytes are currently being evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoelectron microscopy (IEM). Indirect immunoprecipitation of solubilized, radiolabeled membrane antigens of Ib lymphocytes employing both rat anti-H-2Kk,Dk and rabbit anti-Ib TASA and their respective antisera has demonstrated that both proteins share a 16,000 dalton moiety, thus suggesting the 'altered-self' model at the target cell level. These observations are currently being confirmed by direct observation at the ultrastructural level employing a double-labelling technique. Studies concerning the distribution and valence of the Ib TASA indicate that the antigen is randomly distributed on all malignant lymphocytes and occupies about 1% of the total surface area. Similar analyses of the distribution and valence of the H-2Kk,Dk antigens by IEM have been initiated but are too preliminary for comment.

130. LYMPHOCYTES AND HISTOCOMPATIBILITY ANTIGENS

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Recently, procedures have been developed for the selective enrichment (positive selection) and depletion (negative selection) of alloantigen-reactive lymphocytes. These procedures will be applied to two questions concerning the biological function of gene products of the major histocompatibility complex

(MHC) and the high frequency of MHC alloantigen reactive T lymphocytes: (1) The extent of involvement of MHC gene products in the induction and expression of certain T cell mediated immune functions, namely cytotoxicity, delayed-type hypersensitivity and helper responses for B cells; and (2) the possible involvement of anti-MHC receptors present on the majority of T cells in the specificity of triggering with conventional antigens.

These questions have a direct bearing on the mechanism of T-cell stimulation by antigen and on whether T cells possess multiple specificities for antigen.

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131. VIRAL DEREGULATION OF H-2D SYNTHESIS - ROLE IN LEUKEMIA

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There is increasing evidence for the presence of type-C viral genetic information in all mammalian cells. Some of the genetic loci involved in regulating expression of viral information have been well characterized. There is, however, a lack of understanding of the molecular mechanisms involved. The primary thrust of this proposal will address itself to understanding the mechanism of action of H-2 linked genes in conferring resistance or susceptibility to virus-induced leukemia. Two model systems will be investigated: (1) H-2-linked control of radiation leukemia virus (Rad LV)-induced neoplasia, and (2) regulation of cell- and humoral-mediated immunity to AKR tumor cells by H-2 linked genes. Present understanding of the role of H-2 linked genes in conferring resistance to murine leukemia viruses is in a primitive state. The currently proposed studies will broaden our knowledge of such genetic mechanisms by examining their immunological as well as nonimmunological aspects. The specific immunologic studies proposed will examine the type of cells mobilized during the immune response; the determinants present on such cells that mark them as a subclass of lymphocytes; the type of humoral- and cell-mediated responses elicited (e.g., cytotoxic antibody, neutralizing antibody, etc.); the antigenic determinants eliciting such a response; and the association of these determinants with detectable H-2 gene products. Additional studies will examine the notion that susceptibility to viral leukemia results from loss of H-2 and/or virus-associated antigens from virus infected and/or transformed cells, enabling such cells to escape immunological destruction; these studies will also examine H-2 effects on virus assembly.

132. STUDIES ON THE H-2 LINKED SS PROTEIN AND HUMAN C4

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This proposal is directed toward a study to determine the complete amino acid sequence of the fourth component of human complement. This molecule which has a molecular weight of 210,000 and consists of three polypeptide chains is a central molecule in the classical complement cascade. It is of additional interest because of its linkage to the major histocompatibility complex.

133. A NEW ENZYME SPECIFIC FOR HISTONE H2A MODIFICATION

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No summary has been provided to the Smithsonian Science Information Exchange.

134. HISTOCOMPATIBILITY ANTIGENS IN DEFINED MEMBRANES

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The overall objective is to define the chemical and physical parameters which regulate immune cellular recognition. Plasma membrane vesicles obtained from tumor target cells are being characterized for enzyme activities, extent of glycosylation of H-2 proteins, asymmetric orientation, and interaction with specific antibodies. We will reconstitute purified histocompatibility antigens into defined lipid systems which mimic the environment of the naturally occurring lipids in the plasma membrane. We will test these reconstituted antigens for their ability to inhibit recognition between a specific cytotoxic T cell and its target cell or to act as targets and be lysed directly by cytotoxic T cells.

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135. MEMBRANE BIOGENESIS IN NORMAL AND MALIGNANT CELLS

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The objective of the proposed research is to utilize a genetic approach in combination with a biochemical, a cell biological, and a cell-engineering approach to examine the mechanisms involved in the regulation of plasma membrane biogenesis, primarily in normal but also in malignant cells. The system that is being studied consists of two membrane glycoproteins, denoted D and K, whose structures are specified by the major histocompatibility locus of the mouse, the histocompatibility-2 region on chromosome 17. Lactoperoxidase-catalyzed iodination and sodium borotritide reduction of cells treated in situ with glactose oxidase are used to label the D and K proteins accessible on the plasma membrane of macrophages, lymphocytes, and hepatocytes of different inbred mouse strains. Metabolic labeling with fucose, mannose or methionine is used to label the total cellular complement of these proteins in the different strains. Cell fractionation methods, in combination with the differential labeling techniques, are used to localize and quantitate the D and K glycoproteins among the different membrane systems of the mouse cells. The D and K glycoproteins are being isolated from these membrane systems, using specific allo and monoclonal antibodies; they are analyzed and quantitated by two-dimensional polyacrylamide gel electrophoretic techniques. Using these types of analyses, we have shown differential expression of these two H-2-encoded polypeptides in (1) different tissues of the same mouse strain and (2) in the same tissue of different mouse strains. The genetic and biochemical basis for these differences will be examined. Attempts will also be made to differentially modulate the D and K polypeptides in order to trace the intracellular localization and route of biogenesis of the perturbed glycoprotein. As part of these studies, attempts are being made to alter the expression of the D and K polypeptides and to change the histocompatibility-2 phenotype of mouse cells by (1) transferring specificities from one cell to another, using membrane reconstitution and fusion methods and using gene transfer techniques. The expectation is that the above approaches will yield important insights into the genetic control of expression of these membrane glycoproteins, their mode of biogenesis and turnover, and their metabolic function within the cell.

136. MEMBRANE ANTIGEN ORGANIZATION IN TUMOR IMMUNITY

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T lymphocytes from animals immune to synegeneic simian virus 40 (SV40)-induced tumors recognize both virus-induced and histocompatibility-2 (H-2) antigens on SV40 transformed target cells. Evidence presented here suggests that T cells bind to a complex between H-2 and the viral antigen on the tumor

cell, and formation of this complex is necessary, if not sufficient, for induction of cytolytic T cells and for in vitro tumor destruction by T cells. In the work proposed, we will investigate the formation of antigen complexes in the tumor cell plasma membrane and the relationship between complex formation and immune T cell recognition.

Co-capping of tumor-associated antigens with mouse H-2 antigens, reported by this and other laboratories, also indicates physical associations among plasma membrane molecules. We have recently found that antigens which co-cap can also be extracted from the membrane as specific complexes. Both the murine leukemia virus gp71 and a carcinofetal antigen, Ag I, are associated with the H-2 antigens following solubilization of L cell membranes with low concentrations of non-ionic detergents. We will study the requirements for formation of these complexes and the relationship between complex formation and endogenous virus production.

We will establish a model system for T cell recognition of foreign proteins expressed on syngeneic cells. This model will allow quantitative evaluation of immune recognition and manipulation of the immune response to cell surface antigens.

137. STRUCTURES OF HISTOCOMPATIBILITY-2 MEMBRANE ANTIGENS

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The objective of this proposal is to characterize the structures of H-2 antigens. This study will focus primarily on the conformations of the membrane-bound glycoproteins coded for by H-2k and H-2K regions of the major histocompatibility complex of the mouse.

The research plan will be directed in essentially four areas: 1) Several methods will be tested for improving the yields and separation of H-2b antigens as presently isolated in highly purified form from EL-4 cells by a purification scheme developed, in part, by the Principal Investigator. The aim here is to purify H-2b antigens from this lymphocytic leukemia-derived cell line for structural examination by conformationally sensitive spectroscopic probes, such as circular dichroism and fluorescence, and by amino acid composition and sequence analysis. 2) In an extension of current studies by the Principal Investigator, various molecules with public antigenic specificities of the H-2.28 family will be compared by tryptic peptide map analysis. The aim is to clarify the structural basis of this complex family of antigens which has recently led to the discovery of a new class of H-2D antigens. 3) The nature of the antigenic sites of H-2 antigens will be explored by chemical reagents which alter serological reactivity. Peptides protected from modification by specific alloantibody, and therefore presumably in or near antigenic sites, will be identified by tryptic peptide mapping techniques.

BIBLIOGRAPHIC REFERENCES: Sears, D.W. and Polizzi, C.M. 1980. Biochemical evidence for a separate, MHC-linked locus encoding H-2.28 antigens. Immunogenetics (in press). Sears, D.W., Young, S., Wilson, P.H. and Christiaansen, J.E. 1980. Structural characterization of H-2L alloantigens: I. Complete tryptic peptide map analysis of H-2La. J. Immunol. (in press).

138. STRUCTURAL STUDIES OF THE PRODUCTS OF THE H-2 COMPLEX

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The overall objective of the research proposed in this application is the elucidation of the detailed structure of two sets of gene products encoded by the H-2 complex, the major histocompatibility complex (MHC) of the mouse. The gene products to be studied, the H-2K and Ia antigens, are both highly polymorphic sets of glycoprotein molecules integrated in the lymphocyte plasma membrane. The studies proposed in this application should lead to a thorough knowledge of the primary structure of both the protein and carbohydrate portions of these antigens. For the H-2K antigens, the proposed studies involve large scale biochemical purification, amino acid sequence determination, and carbohydrate structure determination. For the Ia antigens, biosynthetic radiolabeling will be combined with immunochemical purification; the materials thus isolated will have their amino acid sequences and carbohydrate structures determined by radiochemical methods. Comparative analysis of amino acid sequence data (employing data obtained in other laboratories as well as our own) will be exploited to try to deduce the antigenically significant portions of these molecules. In addition, experiments will be carried out which approach this problem directly, either through the use of tryptic or cyanogen bromide fragments (of known structure) to inhibit the binding of alloantisera to intact antigens, or through the use of alloantisera to block reaction of protein modification reagents with the antigenic site.

Cross-linking studies will be carried out to try to elucidate interactions of H-2 and Ia antigens with other molecules in the lymphocyte plasma membrane. Finally, experiments will be undertaken to try to establish the molecular function of the H-2 antigens in 'H-2 restricted' anti-viral cytotoxicity; and to examine the function(s) of the Ia antigens in I region regulated antigen presentation systems in vitro.

BIBLIOGRAPHIC REFERENCES: John H. Freed. Study of Immunoprecipitated Gene Products from the H-2 Complex by Hydroxylapatite Chromatography in the Presence of Sodium Dodecylsulfate. Molecular Immunology 17:453-462 (1980).

139. SOMATIC CELL GENETICS OF CELL SURFACE ANTIGENS

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The aim of the proposed research is to isolate and characterize H-2 antigen variants. The variants will be isolated from heterozygous mouse leukemia cells, using two immuno-selective agents: (1) anti H-2 antisera and complement and (2) cytolytic T cells. Survivors of the selection will be characterized by a combination of biochemical, genetic and immunological techniques. Using this approach, we intend to achieve the following goals: 1. In the past few years, we have isolated several variants using antisera. The genetic mechanism involved is not clear, but mitotic recombination resulting in homozygosity for the expressed antigens is a possibility. Experiments designed to test this possibility are described. 2. We will attempt to determine the nature of the T-cell target antigen by establishing a selection system to obtain resistant variants using cytolytic T cells. By studying the nature of the defect in the cells resistant to lysis, we hope to be able to gain insight into the identity of the target antigens(s) of T cells.

3. Using an anti-H-2K kappa hybridoma as the immunoselective agent, we have isolated what appears to be a structural mutant. We will attempt to define the nature of the mutation in this variant. Other variants will be isolated using the same and other hybridomas against H-2K kappa to determine the nature of antibody reactive sites on the H-2 mutants.

140. CELL LINE MUTANTS IN STRUCTURE OR EXPRESSIONS OF H-2 ANTIGENS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

141. CHEMISTRY AND DEVELOPMENT OF TRANSPLANTATION ANTIGENS

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Cell surface antigens and especially mouse MHC antigens, H-2, will be studied as probes for the structure of membranes. Lateral diffusion of H-2 will be examined in single cells in order to understand how lateral diffusion rates are regulated by cells. Expression of HLA antigens in trophoblast cell membranes will be examined serologically and biochemically in order to understand the mechanisms by which these antigens are masked in development, thus protecting the fetus from rejection by its mother. Other embryo antigens, defined by teratocarcinoma cell

surfaces, will be studied for their importance in early development.

BIBLIOGRAPHIC REFERENCES: Lafuse, W. and Edidin, M. Influence of the mouse major histocompatibility complex, H-2, on liver adenylate cyclase activity and on glucagon binding to liver cell membranes. Biochemistry 19: 49-54 (1980). Edidin, M., Wei, T., and Holmberg, S. The role of membrane potential in determining rates of lateral diffusion in the plasma membrane of mammalian cells. Annals of the New York Academy of Sciences 333: 1 (1980).

142. GENETICS AND BIOCHEMISTRY OF MOUSE ALLOANTIGENS

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I plan to investigate a segment of mouse chromosome 17 situated near the major histocompatibility complex (MHC). The objectives are to elucidate the organization of genetic information; define the extent of polymorphism and/or number of loci encoding cell surface antigens in this region; determine the expression of these antigens on various populations of lymphocytes in order to identify markers of functional subsets of lymphocytes; then investigate the biochemical nature of these antigens to determine their evolutionary relationship. In order to accomplish this, I will initiate experiments to search for genetic recombination in this region. These and other recombinants will be serologically analyzed by cross-immunization and absorption of antisera. The biochemical characteristics will be established by immunoprecipitation of antigen and analysis by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), peptide mapping and partial N-terminal amino acid sequence analysis.

143. BEHAVIOR OF WEAK TRANSPLANTATION ANTIGENS

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Primary attention is being devoted to the behavior of grafts which are only incompatible with respect to weak transplantation antigens in mice and rats. Under certain conditions these grafts can evoke an immune response, while under other conditions they may induce unresponsiveness. The basis for these different responses is being studied. More specifically the projects include investigating the behavior of H-Y incompatible grafts (of various types, e.g., skin, parathyroid, islets of Langerhans), as well as those incompatible for putative skin-specific antigens. Studies are also aimed at determining the genetic basis for the expression of, and response to, H-Y antigen in rats. Finally, some attention is also being devoted to elucidating the mode of action of some coat color determinants of the mouse.

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144. BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF TRANSPLANTATION ANTIGENS

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The purpose of this work is to examine the chemical, biological and immunological properties of histocompatibility antigens (H-2) and tumor specific transplantation antigens (TSTA). The approach is to purify the molecules expressing these antigens and determine their properties at each stage of the purification. These properties include: 1) Ability to induce in vivo transplantation rejection and humoral response, 2) ability to stimulate lymphoid cells in vitro, 3) chemical properties with particular emphasis on how these molecules bind to biological membranes, 4) the metabolism of these antigens in normal and neoplastic cells. The rationale for this approach is that through purification of these antigens one can determine if there are any structural similarities which may explain their common biological activity, i.e., transplantation rejection. Furthermore, knowledge of the biological properties of these molecules will provide a rational basis for using them in in vivo therapeutic protocols. Finally, when they are purified, the chemical structure of these antigens will aid in the understanding of the organization of the genes which encode them.

BIBLIOGRAPHIC REFERENCES: Law, L.W., DuBois, G.C., Rogers, M.J., Appella, E., Pierotti, M.A., and Parmiani, G.: Tumor rejection activity of antigens isolated from the membranes of a methylcholanthrene-induced sarcoma, C-1, bearing alien H-2 antigens. Transplantation Proceedings 12: 46-50, 1980. Rogers, M.J., Pierotti, M.A., Parmiani, A., Appella, E.: Alien H-2 antigens (H-2k) appearing on a methylcholanthrene induced tumor (H-2d). Biochemical characterization. Transplantation Proceedings 12: 38-45, 1980.

3. CHARACTERIZATION OF IA ANTIGENS

145. PRODUCTION OF MONOCLONAL ANTISERA AGAINST MOUSE IA ANTIGENS

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OBJECTIVE: To establish hybrid cell lines in long-term culture which synthesize and secrete antibody directed against mouse H-2-linked immune respone (I) region associated (Ia) histocompatibility antigens.

APPROACH: A mouse myeloma cell line which secretes an IgG1 product is fused with mouse spleen cells in the presence of polyethylene glycol according to the method of Galfre et al. (Nature 266, 550 (1977)). The spleen lymphocytes are derived from mice previously immunized against various incompatibilities across the mouse H-2 major histocompatibility complex. H-2 congenic mouse strain combinations differing in their I-region are used for these immunizations. Suitable hybrid clones are grown in vivo as an ascites tumor in H-2 compatible recipients to enable the production of monoclonal anti-Ia antisera. These antisera will be used in the future for biochemical, genetic and functional studies of mouse Ia antigens.

PROGRESS: A monoclonal antibody reactive with Ia antigens determined by the I-Ak subregion has been derived from a hybrid clone grown in ascites form. It is present at about 10 micrograms/ml in the ascites, possesses a titre of approximately 1:10 to the 6th power in a radioimmune cell binding assay, immunoprecipitates Ia antigens examined by gel electrophoresis and is presently being tested in functional assays designed to study the genetic control of lymphocyte interaction.

146. MURINE IA MUTANTS - SOMATIC GENETIC AND BIOCHEMICAL STUDIES

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We propose a coordinate somatic genetic and biochemical approach to study a set of cell surface molecules coded by genes within the major histocompatibility complex, the Ia antigens, which appear to play a critical role in the regulation of the immune response. The mouse has been an excellent experimental system for studies of Ia antigens because of the availability of genetically defined inbred and recombinant inbred strains of mice, highly specific anti-Ia alloantisera, and more recently, monoclonal anti-Ia antibodies. The mouse and human major histocompatibility gene systems are similar and information concerning one system may be effectively applied to the other system. Therefore, these genetic and biochemical studies of the murine Ia antigens should contribute to the understanding of human Ia antigens. In particular in the human system, it will be important to understand the role these antigens play in HLA-associated predisposition to diseases, such as rheumatoid arthritis, multiple sclerosis and others. The first step in these studies is to obtain mutants from Ia antigen-bearing cell lines which lack or show abnormal expression of cell surface Ia. Mutant cell lines will be obtained by three different methods of cytotoxic immunoselection: 1) antibody and complement mediated; 2) cell-medi-

ated; and 3) lectin resistant. The mutant cell lines will be characterized for cell surface expression of Ia by fluorescence activated cell sorter (FACS) analysis, genetically by complementation analysis, and biochemically by two-dimensional polyacrylamide gel (2-d PAGE) analysis of cellular proteins. From the analysis of Ia mutants with deficient or abnormal Ia chains, it is hoped that a more complete understanding of the genetics, structure, and expression of Ia antigens will be obtained which will facilitate further understanding of the complex functions of these cell surface molecules in immune responses and disease susceptibility.

147. PRIMARY STRUCTURE OF MHC I REGION ASSOCIATED ANTIGENS

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Proteins encoded by the I region of the major histocompatibility region (Ia antigens) will be isolated from Ia-bearing tumor cells. The cells will be radiolabeled with 3H, 35S and/or 14C amino acids, detergent solubilized and isolated with immunoadsorbants, utilizing monoclonal anti-Ia alloantisera from hybridomas. Protocols for isolating cyanogen bromide and proteolytic fragments from both the alpha and beta chains of the Ia molecules labeled with a variety of 3H amino acids and 35S cys will be initially developed. These fragment isolation procedures will be designed to maximize the recoveries of the radiolabeled products. Once the peptide isolation procedures are established, Ia molecules will be uniformly radiolabeled with 14C, the peptides isolated and sequenced.

BIBLIOGRAPHIC REFERENCES: McKean, D.J., Moosic, J.P., Nilson, A., Hammerling, G.: Biochemical Characterization of Ia Antigens. I. Characterization of the 31K Polypeptide Associated with I-A Subregion Antigens. J. Immunol., October, 1980, in press. Moosic, J.P., McKean, D.J., Nilson, A., Hammerling, G.: Isolation of Three Ia Polypeptides with monoclonal immunoadsorbants. Fed. Pro., 38, 809, 1980.

148. STRUCTURAL STUDIES OF IA ALLOANTIGENS

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The major histocompatibility complex (MHC) of the mouse controls a number of immunologically related functions. The immune response of individuals and inbred strains to a wide variety of synthetic and natural antigens is controlled by dominant genes that map within the I region of the MHC, and have been termed histocompatibility or H-linked Ir genes. The I region also codes for a series of cell surface (Ia) antigens. A number of experiments suggest that Ir genes exert their effect via cell interactions mediated by Ia alloantigenic molecules. With the goal of elucidating the molecular basis by which Ia alloantigens mediate their functions, we propose to subject them to detailed structural studies. We will employ microsequencing methods that have recently been developed, and which have previously been successfully applied to the structural analysis of the H-2K and H-2D gene products. Such studies may provide a framework for correlating the structure of these molecules with their functions.

149. STRUCTURAL AND SEROLOGICAL STUDIES ON IA ANTIGENS

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The Ia alloantigens are the only isolated gene products of the immune response control region (I region) of the mouse major histocompatibility complex (MHC). The molecules bearing Ia alloantigenic determinants have been implicated in the control of immune response function by experiments showing that anti-Ia alloantisera specifically inhibit such functions. Furthermore, the Ia structural genes are arranged so that the Ia molecules could provide a molecular explanation for Ir gene complementation effects. Since Ia antigens may have an active role in the control of immune response, these molecules must be analyzed to see if their structural features can explain how they carry out their proposed function.

The research projected here includes structural, genetic and serological investigation of Ia molecules. Structural analysis will include assessment of variability in primary structure, primarily by peptide mapping, localization of antigenic sites, search for interaction with other cell proteins, and mechanism of assembly and transport of Ia molecules to the cell surface. Genetic analysis will include a survey of available recombinants to further define the arrangement of Ia structural genes. Serological analysis will mainly involve a search for antibodies to unassembled Ia chains, or to all antigenically silent Ia molecules or chains.

BIBLIOGRAPHIC REFERENCES: Cullen, S.E., Rose, S.M. and Kindle, C.S. 1981. Ia antigens: Molecular components in immune regulation? In Current Trends in Histocompatibility. Eds. Reisfeld, R.A. and Ferrone, S. Plenum, New York in press p. 391-413. Cullen, S.E., Shreffler, D.C., Kindle, C.S. and David, C.S. 1981. Identity of molecules bearing murine Ia alloantigenic specificities Ia. 7 and Ia. 22. Evidence for a single type of I-E molecule in homozygotes. Immunogenetics, in press.

150. EXPRESSION OF IA ANTIGENS ON FUNCTIONAL CELL SUBPOPULATIONS

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It has been demonstrated that the T cell proliferative response to Con A and the T cell dependent IgM response to the soluble antigens TNP-KLH, TNP-T,G-(A-L), and TNP-nuclease require the participation of accessory cells. This subpopulation of spleen cells has been shown to be glass adherent, radioresistant, non-T, non-B and to express Ia (I region associated) determinants encoded in I-A and I-E/C. In addition, cells within the splenic adherent cell population are the predominant stimulators of the one-way murine mixed lymphocyte response when responder and stimulator cells differ either at H-2 or the Mls locus. These stimulator cells also bear I-A and I-E/C encoded determinants and their ability to stimulate allogeneic T cells could be inhibited with specific anti-Ia reagents. Similar results regarding Ia antigen expression and functional accessory cell competence were also obtained using a population of purified murine Kupffer cells, the tissue macrophage of the liver.

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151. IA ANTIGENIC SPECIFICITIES IN MOUSE AND MAN

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We propose to study the chemistry, genetics, immunology and physiology of Ia antigenic specificities secreted into serum in a murine model and in man. Studies by other investigators have already demonstrated the importance of these specificities in the MLR, in T-B cell co-operation and in graft rejection. Our studies have already demonstrated the presence of Ia substances in serum and that these can be detected by a rabbit antiserum as well as by the conventional alloantibodies. We have also shown that Ia substances of low molecular weight are secreted by helper T cells after contact with antigen, and that this material is predominantly carbohydrate. We have also made substantial progress with this model in man. We now intend to study in the murine Ia model: a) The carbohydrate chemistry of the Ia specificities: structure of high molecular weight and low molecular weight components and further chemical analysis using enzyme studies; b) to examine secretion in tumour bearing mice (in allogeneic and syngeneic recipients) and after treatment with carcinogenic agents; c) examine the secretion in rats undergoing renal allograft rejection; d) examine secretion in mice under conditions which enable determination of the specificity of the Ia secreted and its relations to antigenic stimulation, e) to determine, in a GVH model, the genetic control of secretion of I region specificities. We also intend to further our genetic studies of H (Ia) - the human equivalent to the Ia specificities and to exploit the demonstrated homologies between murine and human Ia to study the chemistry, immunology and physiology of Ia secretion in man. In particular we wish to determine

whether the measurement of Ia levels is equivalent to monitoring T cell function. Thus far, it seems that it is, but two circumstances require intensive study, a) the rejection of human renal allografts, b) examination of the serum of patients with cancer.

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152. SEROLOGICAL DEFINITION OF T CELL IA ANTIGENS

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The I region of the H-2 complex is known to control a number of immune phenomena, some of which are thought to be strictly T cell functions, such as the MLR, QVHR, and interaction with macrophages, and some of which involve B cells, such as T-B collaboration and antibody responses to defined antigens. Although I region products (Ia molecules) are easily found on B cells, early studies indicated that T cells do bear Ia molecules, and at least one of these antigens is different from those found on B cells. The objective of this project is to study Ia antigens on certain T cell subpopulations by serological and biochemical techniques, some of which will be done as a collaborative effort. For this purpose, the enrichment of three T cell subpopulations will be obtained: an I-J(plus) suppressor cell, an I-J(plus) helper cell, and a presumed I-C(plus) MLR suppressor cell. The enrichment methods for the first two are already published, and it is hoped that similarly simple methods will also yield the third. These subpopulations will be used in the following ways: (1) existing antisera will be used for serological characterization; (2) a collaborating lab will analyze the cell-surface Ia biochemically; (3) antisera will be produced in congenic mice and tested as in number 1; (4) the known functions of the subpopulations will be tested to evaluate the effects of the above antisera.

153. GENETICS AND FUNCTION OF MURINE IA ANTIGENS

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We will continue our work on the generation of lines as assay systems for antigen presentation to determine Ia's role in recognition of antigen by T lymphocytes. We will further examine the role of Ia molecules in skin graft rejection in I region compatible strains.

We will also examine the role of Ia in non-lymphoid tissue such as brain and the potential for an endogeneous lymphoid immune system in the brain. Further we will begin studies on genetic organization at the molecular level of chromosome 17 DNA sequences in collaboration with other laboratories.

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154. GENETICS AND FUNCTIONS OF (H-2 LINKED) I REGION

David, C. S., Krco, C. J., Lafuse, W. P., Christadoss, P., Long, P. M., Wooley, P. H., Gorzynski, T. J., Mayo Foundation, Dept. of Immunology, *200 1st St. S.W., Rochester, Minnesota,* 55901, United States of America

In the recombinant screening program during the past year, we screened approximately 5,000 mice for crossing over, mutation, and genetic variation. We picked up 10 new recombinants. The recombinants are being established as congenic inbred strains before further analysis will be carried out. Two recombinants from the previous year were established as congenic strains and made available to laboratories outside. We extended our studies in gene complementations to show all the possible allelic combinations which can generate new I region gene products capable of antigen presentation. By using an Ia mutant, we were unable to show that Ir genes, MLR determinants, and Ia antigens are coded by the same gene. In the past year, we have also shown that Ia molecules mediate autoimmunity in the case of myasthenia gravis. We have established a model for rheumatoid arthritis in mice and have mapped the genes involved with the I region. Further studies are also currently under way on the genetics of parasite infection, and thyroiditis. We have also found that the immune response to an oligospecific molecule such as hemoglobin could be directed against different determinants on different chains. Some of it might signal a negative effect inducing suppression.

BIBLIOGRAPHIC REFERENCES: David, C.S. Regulation of immune response to MHC-linked genes. Transplantation Proc. 12:8, 1980. Lafuse, W.P., Neely, B.C., and David, C.S. Biochemical studies of mouse Ia molecules with zenoantisera. I. Identification of a second Ia molecule (I-E?) in H-2s haplotype. Immunogenetics 10:311-318, 1980.

C. HISTOCOMPATIBILITY TYPING STUDIES

155. MHC AND CANCER SUSCEPTIBILITY GENES IN MAN

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We proposed to seek cancer susceptibility genes within the major histocompatibility locus on chromosome 6 in patients with various types of cancer and in high-risk cancer families. In order to accomplish this, we proposed to further develop a method of HLA-D typing which utilizes lymphoblastoid cell lines (LCL) instead of peripheral blood lymphocytes from donors who are homozygous at the HLA-D locus. Within the past year, we have further developed this technology and the computer software and interfacing it entails. We also compared this method of HLA-D typing with other methods and found good agreement. When the various alleles at the HLA-D locus were compared as they were represented by our LCL cell panel, it was possible to appreciate that alleles Dw1 and 3 split and overlap in a pattern suggesting new allelic groups: 1a-3, 1b-3 and 'true 3.' The often-questioned allelic group, Dw8, was included within our group 1b-3. HLA typing by serological methods was established in our laboratories as a result of this award and will continue on a cost-effective basis after the award has expired. In addition, a screening program for new HLA antisera was established. The extensive LCL cell panel created for these studies now represents a unique research tool and has recently been utilized in several new ways: 1) To demonstrate that antisera to HLA-DR specifically inhibit growth of such cell lines, 2) to characterize the relationship between the two closely related loci of HLA-D and HLA-DR. During this year, we have critically reevaluated each LCL in our homozygous typing cell panel, deleted many, and added 17 new ones. All new entries to this cell panel will be from consanguineous donors. We have initiated the final phases of the project by contacting various cancer agencies to inform them of our capabilities and interest in typing high-risk cancer families. Several such families have already been typed; however, it is premature to state results.

BIBLIOGRAPHIC REFERENCES: Gatti, R.A.: The Genetics of Cancer. Infectious Disease 26:4-26, 1978.

156. NCI HISTOCOMPATIBILITY TESTING CENTER

Johnson, A. H., Duke University, School of Medicine, Dept. of Microbilogy & Immunology, *Box 3711, Durham, North Carolina,* 27710, United States of America

OBJECTIVES: HL-A phenotyping of approximately 2,500 samples. Maintenance of a panel of cells well defined for HL-A antigens. Assessment of serums for antibodies to HL-A and other antigens by the lymphocyte cytotoxicity assay. Mixed lymphocyte cultures: Perform approximately 400 MLC reactions between lymphocytes from blood samples provided by the P.O. and a panel of donors accessible to the contractor, who have defined alleles at the MLC locus; and perform approximately 100 MLC reactions between pairs of blood samples provided by the P.O.

157. HISTOCOMPATIBILITY COMPLEX AND THE EXPRESSION OF ANTIGEN SYSTEM IN LEUKEMIA

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This is part of a broader project. A summary of this subproject is not available.

158. HISTOCOMPATIBILITY TESTING AND IMMUNOGENETIC STUDIES

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This is part of a broader project. A summary of this subproject is not available.

159. THE MAJOR HISTOCOMPATIBILITY COMPLEX OF THE RABBIT

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The laboratory rabbit has the potential to be a useful non-inbred animal model for transplantation studies; however, the inability to type for the antigens of the major histocompatibility complex severely limits its use at the present time. We are attempting to develop monospecific typing antisera and homozygous typing cells for the major histocompatibility loci. These reagents should provide the ability to haplotype commercially available animals and increase the usefulness of the rabbit as a model system.

BIBLIOGRAPHIC REFERENCES: Tissot, R.G., Lancki, D.W., and Blaesing, M.E. 1979. Homozygous Cell Typing for the Rabbit RLA-D Antigens In Animals From Commercial Breeders. Immunogenetics 8: 509-515. Knight, K.L., Leary, A.L., and Tissot, R.G. 1980. Identification of Two Ia-like Alloantigens on Rabbit B-Lymphocytes. Immunogenetics (in press).

V. TRANSPLANTATION BIOLOGY AND CANCER

A. GRAFT REJECTION AND TOLERANCE

160. INDUCTION, GROWTH AND REPRESSION OF PRIMARY AND TRANSPLANTABLE NEOPLASMS (HOST AND TUMOR CELL VARIABLES) (ABBREV)

Law, L. W., Appella, E., Rogers, M., DeLeo, A., Old, L. J., Hopkins, N., Alaba, O., Parmiani, G., DuBois, G., Chang, K. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Biology Laboratory, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

OBJECTIVES: Major emphasis is placed upon the study of tumor antigens of the transplantation rejection type (TSTA), and of tumor antigens (TA) assayed by in vitro techniques and of the immune responses they evoke. As a corollary to this study the biologic properties in vitro and in vivo of histocompatibility (H-2) antigens are under study. Solubilization and methods of purification of both TSTA and H-2 are under investigation with the ultimate purpose of defining these membrane antigens after purification in physico-chemical, biologic and molecular terms. The role of a B-tropic retrovirus as a vector in the transmission of genetic information of a specific tumor antigen (TSTA of Meth A) is now under study as well as the use of TSTA markers (Meth A) in the hope of defining more clearly the molecular events in transfection studies using DNA of chemically induced sarcomas.

BIBLIOGRAPHIC REFERENCES: Law, L.W., DuBois, G.C., Rogers, M.J., Appella, E., Pierotti, M.A., and Parmiani, G.: Tumor rejection activity of antigens isolated from the membranes of a methylcholanthrene-induced sarcoma, C-1, bearing alien H-2 antigens. Transplantation Proceedings XII: 46-49, 1980. DeLeo, A.B., Jay, G., Appella, E., DuBois, G.C., Law, L.W., and Old, L.J.: Cell surface antigens of chemically induced sarcomas of inbred mice. Transplantation Proceedings XII: 65-69, 1980.

161. ALLOGRAFT REJECTION AND ENHANCEMENT

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The aims of this research proposal have been to investigate host responses toward transplanted tissue allografts, both those involved in the phenomenon of acute rejection by unmodified recipients and those involved in prolonging graft survival in enhanced animals. Our studies have been concerned primarily with assessment of the dynamic and changing relationships between cellular and humoral host mechanisms involved in these processes. Cellular responses have been studied by assessing activity of T lymphocyte populations and subpopulations, both those infiltrating the graft itself and those in peripheral lymphoid tissues. The role of thymocytes in acute rejection and enhancement has been pursued, as evidence of suppressor activity has been noted previously in enhanced animals. Patterns of migration of thymocytes and of T and B lymphocytes are being assessed by double radiolabelling experiments in both unmodified and enhanced graft recipients. Interaction between lymphocytes and macrophages in acute rejection and enhancement is under investigation.

BIBLIOGRAPHIC REFERENCES: Tilney, N.L., Garovoy, M.R., Busch, G.J,, Strom, T.B., Graves, M.J., Carpenter, C.B.: Rejected human renal allografts; recovery and characteristics of infiltrating cells and antibody. Transplant. 28(5):421, 1979. Tilney, N.L.: Host responses and immunological suppression. Dialysis and Transplantation. 9:114, 1980.

162. MECHANISM OF DELAYED HYPERSENSITIVITY AND TUMOR GRAFT REJECTION

Rapp, H. J., Zbar, B., Ashley, M. P., Sukumar, S., Sugimoto, T., Hartley, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cellular Immunity Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

It is the purpose of this project to elucidate the mechanism of the immunologic rejection of tumor grafts and to determine the mechanism of tumor suppression following immunostimulation with BCG. Current areas of investigation are: evaluation of the specificity of immunization evoked by living BCG and fibrosarcoma cells and development of methods for elimination of microscopic metastases remaining after surgery.

BIBLIOGRAPHIC REFERENCES: Zbar, B., Canti, G., Rapp, H.J., Ashley, M.P., Sukumar, S., and Bast, R.C., Jr. Immunoprophylaxis of syngeneic methylcholanthrene-induced murine sarcomas with Bacillus Calmette-Guerin and tumor cells. Cancer Res. 40: 1036-1042, 1980.

163. IDENTIFICATION OF CELL TYPES RESPONSIBLE FOR REJECTION OF GRAFTS BEARING WEAK ANTIGENS

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OBJECTIVE: To determine the cell types responsible for rejection of grafts bearing weak antigens.

APPROACH: Weak transplantation antigens are thought to mimic tumor antigens. We have, therefore, explored immune responses to a weak histocompatability antigen, the male specific antigen, H-Y. We need to determine which factors influence these responses in the hope that this information will be applicable to the control of responses to tumor antigens, and that better ways of manipulating anti-tumor responses in vivo can thus be devised.

PROGRESS: Two main T cell responses to H-Y antigen have been found: (1) The generation of H-2 restricted cytotoxic responses in vitro and (2) the rejection of grafts in vivo. The genetics of response (1) are very complex, and imply control mediated via antigens of the major histocompatability complex (MHC) both in mouse and in man. The genetics of response (2) are less complex but it is controlled by MHC genes and it seems clear from studies with H-2 congenic and recombinant mice that response (1) and response (2) are mediated by different cell types. We know at least the identity of the effector cell in the in vitro response (1), but there is scant information about the effector cell type(s) involved in graft rejection.

164. CELL-MEDIATED HYPERACUTE REJECTION

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Cell-mediated hyperacute rejection (CMHAR) of allogeneic mouse skin is an experimental model which differs from (probably serum-mediated) hyperacute rejection of transplanted viscera (in man or experimental animals) with respect to kinetics and immune pathways. There is a resemblance with respect to prominent involvement of the vasculature of the transplanted tissue, and the marked acceleration of the rejection process. The present study is aimed at elucidating events at the target site, and at dissecting the immune pathway(s) involved in bringing CMHAR about. This involves the identification of in vivo and in vitro analogs reflecting parallel pathways. Specific antisera (e.g., anti-Ly.t sera) are being used to characterize the effector cell(s) participating in CMHAR, in comparison to other, not hyperacute forms of rejection.

BIBLIOGRAPHIC REFERENCES: E. J. Eichwald et al. Analogs of the Murine White Graft Reaction. Cell Imm. 43:398, 1979. M. Emam et al. Transfer of cell-mediated hyperacute rejection reaction. The role of active sensitization. Cell Imm. 49:426, 1980.

165. MIXED LEUKOCYTE CULTURE AND TYPING FOR LIVER ALLOGRAFT REJECTION IN DOGS

Fortner, J. G., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

166. GRAFT REJECTION

Gale, R. P., University of California, Los Angeles Campus, School of Medicine, Dept. of Microbiology & Immunology, *2025 Zonal Ave., Los Angeles, California,* 90024, United States of America

This is part of a broader project. A summary of this subproject is not available.

167. STABLE GRAFT-HOST TOLERANCE AND GRAFT-VERSUS-HOST DISEASE (HUMAN)

Storb, R. F., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

This is part of a broader project. A summary of this subproject is not available.

168. TOLERANCE OF BONE MARROW GRAFTS

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The overall objective of our grant is to define the effect of genetic and environmental factors on the survival time of x-irradiated mice undergoing graft-vs-host (GVH) after reconstitution with H-2 disparate bone marrow cells. One aim was to establish the number of independently segregating genes which controlled products previously assigned to the presumed single gene, Mls. Clarification of the ambiguities of Mls is an important point in our studies of the suspected ability of Mls products to modulate GVH induced by H-2 disparite cells.

BIBLIOGRAPHIC REFERENCES: Click, R. E., Tolerance of histoincompatible marrow grafts, Transplant. Proc. 11: 490, 1979.

169. MECHANISMS OF ALLOGRAFT TOLERANCE

Wright, P. W., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

The purpose of the proposed investigation is to better characterize the reactive lymphoid cells and serum blocking factors in operationally tolerant animals. This will be accomplished by the development of specific anti-rat T-cell reagents and by the identification of functionally distinct subpopulations of rat lymphoid cells using quantitative MLC and CML assays. Studies will be undertaken in H-2 recombinant mice to establish which antigens determine immunologic reactivity in tolerant hosts and to define which genetic mechanisms control responsiveness to these antigens. In vivo mechanisms of importance in transplantation tolerance will also be evaluated. These studies will critically test the hypothesis that subpopulations of lymphoid cells in tolerant animals may demonstrate different patterns of unresponsiveness to alloantigens.

B. GRAFT VERSUS HOST REACTIONS

170. MECHANISM OF GVHD POST MARROW GRAFTING

Tsoi, M., Fred Hutchinson Cancer Research Center, Division of Medical Oncology, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

Marrow transplantation from HLA identical siblings has been used with increasing success for the treatment of otherwise fatal malignant and non-malignant hematological diseases. A number of patients are surviving for up to 8 years leading normal lives. Many patients, however, have died either from recurrent disease or, more frequently, from graft-versus-host disease (GVHD) and associated infectious complications. Furthermore, 20% of long-term survivors develop chronic GVHD and associated immunologic deficit. It is the objective of this proposal to investigate the mechanisms involved in acute and chronic GVHD in patients with marrow grafts.

Using two sensitive cell-mediated cytotoxicity assays we have shown marked in vitro cytotoxicity against patient fibroblasts caused by a lymphocyte subpopulation in acute GVHD patients. On the other hand, we have demonstrated nonspecific suppressor activity of mononuclear leukocytes in chronic GVHD patients, inhibiting donor lymphocyte response in the blastogenesis assay. Long-term stable survivors, however, have neither specific cytotoxicity nor nonspecific suppressor activities. We propose to study the effector mechanism of acute GVHD and also the suppressor mechanism in chronic GVHD. Sequential and comparative studies will be undertaken to understand the relationship of these cells to acute and chronic GVHD. Special effort will be devoted to the isolation of the cytotoxic and the suppressor cells. While the former cells may be isolated by immunoabsorption on antigen-specific fibroblasts, both cell populations may be isolated by various column chromatography, selective rosetting, determination of density gradient and sedimentation velocity methods. These cells will be characterized on the basis of surface marker properties. Soluble suppressor factors, if present, will be isolated and characterized by biochemical methods. The possibility of producing heteroantisera against the purified cells and soluble suppressor factor will be considered.

171. INTERFERON FOR CMV INFECTION, LEUKEMIC RELAPSE AND GVHD

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Efficacy of human leukocyte interferon in the prevention of CMV infection, relapse of leukemia, and graft-versus-host disease after marrow transplant for acute lymphocytic leukemia in remission is being studied in a randomized protocol.

Initial studies have identified a dose that seems both to be safe and to have measurable biological effects when given every 3 days to marrow transplant patients. Preliminary analysis suggests, for example, that the natural killer (NK) activity of circulating lymphocytes is increased in treated patients.

Data on the clinical efficacy of interferon should be forthcoming in the next 1-2 years.

172. CLINICAL AND EXPERIMENTAL STUDY OF IMMUNOLOGICAL FUNCTION AND GRAFT-VERSUS-HOST DISEASE IN BONE MARROW TRANSPLANTATION

Humphrey, J. H., Gordon Smith, E. C., University of London, Royal Postgraduate Medical School, Dept. of Immunology/Haematology, *DuCane Rd., c/o Hammersmith Hospital, London W12 0HS, England, United Kingdom*

Patients receiving bone marrow allografts for aplastic anemia or acute leukemia, or bone marrow autografts for acute leukemia in relapse (accompanied by cytotoxic drug and/or irradiation) are monitored for development of GVH disease and for in vitro reactivities with preserved recipient lymphocytes taken before grafting. The aim is to discover whether GVH disease can be predicted, and what factors determine its onset or remission. Empirical observations are made on absorption of

bacterial endotoxin from the gut, on interferon production and on various routine immunological parameters.

Mild or severe GVH is produced in allogeneic and congeneic rats subjected to bone marrow transplantation, with the aim of discovering the cells responsible for GVH disease and the efficacy of antibodies against helper and/or suppressor T-cells in preventing or curing GVH disease, and of studying the immunological effectiveness of surviving host macrophages and monocytes derived from donor bone marrow.

173. GRAFT-VERSUS-HOST DISEASE AND ITS TREATMENT (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

174. GRAFT-VERSUS-HOST REACTION AND DISEASE (HUMAN, RAT)

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This is part of a broader project. A summary of this subproject is not available.

175. COMPLEMENT STUDIES IN GRAFT-VERSUS-HOST DISEASE

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This is part of a broader project. A summary of this subproject is not available.

176. GRAFT-VERSUS-HOST-DISEASE

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This is part of a broader project. A summary of this subproject is not available.

177. EFFECT OF IMMUNOSUPPRESSIVE THERAPY AND GRAFT-VS-HOST DISEASE

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This is part of a broader project. A summary of this subproject is not available.

178. SUPPRESSION OF TUMOR GROWTH BY GVH SERUM FACTORS

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This is part of a broader project. A summary of this subproject is not available.

179. CONTROL OF GRAFT-VERSUS-HOST DISEASE

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No summary has been provided to the Smithsonian Science Information Exchange.

180. ANTI-TUMOR POTENTIATION AND SPONTANEOUS LYMPHOMAS IN THE MURINE GVH SYSTEM

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The relationship between immune disorders and lymphoid neoplasia is unknown but patients with primary immune deficiencies have a high incidence of malignancy. Similarily, the reasons why tumors grow in the face of an array of apparent anti-tumor immune mechanisms (specific and non-specific) is not clear. The murine graft-versus-host (GVH) reaction, induced by the injection of parental strain cells into a genetically tolerant F1 hybrid, provides a model in which: (1) an array of immune disorders occur, (2) a high incidence of spontaneous lymphomas occur and (3) differences in growth of transplanted tumors can be demonstrated. Therefore we propose to use the murine GVH model to investigate parameters that may influence the induction of spontaneous lymphomas as well as to investigate mechanisms that may enhance or retard the growth of an established tumor.

To study factors that influence the induction of primary tumors we shall investigate the following parameters in relation to the appearance of GVH induced spontaneous lymphomas: (a) lymphoid cell origin of the lymphoma, (b) altered non-specific cellular cytotoxic mechanisms including NK cell activity and tumoricidal macrophages, (c) pathological thymus changes resulting in defective T cell immunity, (d) the production of suppressor cells, and (e) the genetic disparity required to induce spontaneous lymphomas as well as the various immune disorders.

To investigate factors that influence the growth of established tumors we shall transplant tumors into mice at various times post-GVH and will monitor tumor growth in relation to non-specific anti-tumor cellular mechanisms including NK cell activity, interferon production, mononuclear phagocyte function and prostaglandin production. In addition we will monitor conventional immune parameters, i.e., antibody production, allograft reactivity and the mitogen response to T and B cell mitogens.

The results of these studies should increase our understanding of the mechanisms potentiating anti-tumor resistance as well as providing further insight into the possible factors responsible for the induction and growth of spontaneous lymphomas.

181. IMMUNOGENETIC REQUIREMENTS FOR THE DEVELOPMENT OF IMMUNOBLASTIC LYMPHADENOPATHY

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OBJECTIVE: A classical animal model for induction of immunoblastic lymphadenopathy is the chronic graft-versus-host reaction in F1 hybrid mice injected with parental strain spleen cells. Previous work with this model has established that for induction of lymphadenopathy the injected parental spleen cells must be able to react to incompatible MHC structures of the F1 hybrid. It is our objective to test if T lymphocytes among the parental spleen cells are the critical donor cell population. If this is so, the possible roles of T helper and T suppressor cells in induction and prevention, respectively, of lymphomagenesis will be studied. This will be complemented by the further determination of the intra-H-2 incompatibilities against which the T helper and T suppressor cell subpopulations react.

APPROACH AND PROGRESS: In order to investigate if parental T lymphocytes are the only donor cell population capable of triggering malignant lymphomas in F1 hybrid recipients, several groups of (BALB/c x A/J)F1 hybrids have been injected with equal or mutually comparable numbers of living BALB/c cells from different sources: a) normal BALB/c spleen cells; b) BALB/c spleen cells that were enriched in T cells after passage over a nylon wool column; c) BALB/c spleen cells depleted of T cells by treatment with rabbit-anti-mouse T cell serum and complement; d) BALB/c spleen cells treated with normal rabbit serum and complement; e) normal BALB/c bone marrow cells.

These groups of mice will be autopsied at 10 months after induction of GVHR and histologic examination and immunofluorescence tests on frozen sections using labelled antisera against mouse-Ig and mouse-T-cell antigen will be performed. It will be attempted to prepare cell fractions varying in number of T suppressor and T helper cells to investigate which of the T cell subpopulations of the parental donor strain are responsible for GVHR-induced lymphomagenesis. The possible methods to be used here will include the following: a) preparation of antisera to Ly antigens, and b) preparation of antisera to I-J antigens on T suppressor cells.

182. EFFECTS OF GRAFT-VERSUS-HOST REACTIONS ON CELL-MEDIATED IMMUNITY

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The intravenous injection of F1 hybrid mice on the C57BL/10 background with parental spleen cells resulted in a loss in the ability of the F1 mice to generate T-cell mediated cytotoxic responses in vitro to TNP-self and alloantigens. The total abolition of response potential occurred within 7 days of injection, persisted for at least three weeks, and could be induced with as few as 2 times 10 to the 6th power spleen cells. The loss of response potential depended on the H-2 type of the parental cells, since H-2k,a spleen cells induced unresponsiveness, whereas H-2b spleen cells did not. The phenomenon is: (a) strain-specific (possibly depending on the C57BL/10 genetic background), since strains of mice with other genetic backgrounds injected with parental cells do not exhibit the loss of immune potential; and (b) dependent on a reaction against F1 alloantigens by grafted parental cells (GVH), since loss of immune activity was associated with enlarged F1 host spleens. Heterogeneous populations of suppressor cells were found to be responsible for loss of immune potential.

BIBLIOGRAPHIC REFERENCES: Shearer, G. M. and Polisson, R. P.: Mutual recognition of parent and F1 lymphocytes: Selective abrogation of cytotoxic potential of F1 lymphocytes by parental lymphocytes. J. Exp. Med. 151: 20-31, 1980. Shearer, G. M., Polisson, R. P., Miller, M. W., and Cudkowicz, E. M.: Genetic control of natural resistance to graft-versus-host-associated suppression of T cell-mediated lympholysis. In Skamene, E. (Ed.): Proceedings of International Symposium on genetic control of natural resistance to infection and malignancy, Montreal, Quebec, March 18-20, 1980, in press.

183. CHRONIC GRAFT-VERSUS-HOST DISEASE IN THE RAT RADIATION CHIMERA

Beschorner, W. E., Tutschka, P. J., Johns Hopkins University, School of Medicine, Dept. of Pathology, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

Chronic graft-versus-host disease (GVHD) is a frequent and often fatal sequela of human allogeneic bone marrow transplantation with features of scleroderma, Sjogren's syndrome and chronic active hepatitis. We have observed a very similar disease developing spontaneously in rat radiation chimeras. We have been able to induce chronic GVHD rapidly and consistently by two alternative methods: sublethal irradiation of chimeras followed by a booster bone marrow transplant and by adoptive transfer of chimera marrow and lymphocytes into third party irradiated recipients. Histology reveals dense dermal collagenosis, lymphoplasmacytic infiltrate and injury of the salivary and lacrimal gland ducts and chronic hepatitis with bile injury. Preliminary immunopathological studies suggest the presence of both allogeneic and organ specific antibodies.

This model has excellent potential for the study of the pathogenesis of chronic GVHD, a preclinical model for modes of therapy and prevention of chronic GVHD, and of probable value for studying some autoimmune disorders. We plan to thoroughly characterize the histopathology, distinguishing the disease from effects of aging, irradiation and infections. Immunopathological characterization will include definition of humoral and cellular components, antigen specificity of acid eluates of target organs and separation of the reactions against histocompatability antigens for reaction against organ specific antigens.

184. ROLE OF MURINE C-TYPE ONCORNAVIRUSES IN THE PATHOGENESIS OF HOST-VERSUS-GRAFT DISEASE

Cross, S. S., Virginia Commonwealth University, Medical College of Virginia, Dept. of Pathology, *1200 E. Broad St., Richmond, Virginia*, 23298, United States of America

Host-versus-graft (HVG) syndrome is the fatal complex of lesions with T(negative) B(positive) immunodeficiencies which may be induced in parental strains of mice perinatally inoculated with related F1 hybrid spleen cells. Manipulation of F1 cell dosage and time exposure produces 1) acute HVG disease and death in 30 days or less (exposure of newborn to F1 cells on day of birth and again on day 7), or 2) long-term survival of six months and development of lymphomas (single exposure on day of birth).

Recent work has indicated that aberrant host responses, histoincompatible cells and murine leukemia viruses (MuLV) are essential for the development of acute HVG syndrome of RFM/(T6xRFM)F1 chimeras. The work also indicates that exogenous MuLV has a significant role and may enhance the expression of new C-type viruses. These viruses have been isolated in mink cells from RFM/(T6x RFM)F1 chimeras. They have biological properties of both ecotropic and xenotropic MuLVs. Viruses are recovered after long-term co-cultivation with the exception of the lymphomas, and these appear in early transfer passages. Immune responses to these C-type viruses and ecotropic MuLV are elevated in RFM/(6xRFM) F1 mice as compared to normal controls. In the proposed studies we will determine the immunoglobulin specificity of this immune reaction to attempt to define both the antigens and antibodies related to immune complex formation in the kidneys and the hypergammaglobulinemia.

A major proposal is to examine the biological and biochemical properties of the new C-type RNA viruses and determine their origin.

Data shows that the RFM/(T6xRFM)F1 chimera has the pattern of virus expression found in the (T6xRFM)F1 hybrid. There is restriction of virus replication in the thymocytes.

The RFM(T6xRFM)F1 chimera presents a complex model for studies of MuLV restriction, enhancement, and effects of exogenous MuLV in allogenic reactions. The model should provide some practical and logical methods for searching for C-type viruses in human leukemias.

C. BONE MARROW TRANSPLANTATION

185. MARROW TRANSPLANTATION IN APLASTIC ANEMIA AND LEUKEMIA

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This program project is a coordinated multidisciplinary program of clinical and basic research into the biology of marrow transplantation as manifested in patients undergoing marrow transplantation for aplastic anemia and leukemia.

The studies in progress are designed to assess the contribution of various genetic, immunologic and hematopoietic functions to engraftment, graft-vs.-host disease or increased susceptibility to infection. Immunogenetic analyses are designed to distinguish those alloantigenic systems within and outside the major histocompatibility complex which must be common to donor and host if engraftment is to be regularly achieved and severe graft-vs.-host disease evaded. Studies of hematopoietic function are principally concerned with the biological processes which enhance or limit regeneration of normal hematologic function in the transplant recipient. Similarly, studies of the process of immune reconstitution in the marrow transplant recipient are designed to determine those functions or dysfunctions of the lymphoreticular system of the host (including lymphocytes, phagocytes, and the organs in which they develop) which may limit engraftment and functional reconstitution and, conversely, those of the marrow graft which influence the development and clinical expression of graft-vs.-host disease or complicating infections.

The patients participating in these studies are individuals with aplastic anemia or leukemia who receive transplants of marrow after treatment with standardized experimental courses of radiotherapy and/or chemotherapy for cytoreduction or immunosuppression. The clinical and experimental results of these studies will then be used as a reference base for the development and selection of new therapeutic protocols for randomized trials.

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186. USE OF BONE MARROW TRANSPLANTS IN THE TREATMENT OF ACUTE LEUKEMIA

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OBJECTIVE: To explore the value of bone marrow transplantation in the treatment of acute leukemia, especially acute myeloid leukemia in first remission.

APPROACH: The protocol is that used in Seattle, i.e., high dose cyclophosphamide and whole-body irradiation but with two main differences: a) the dose-rate of irradiation will be slow (2.5 rad/min) and unfractionated; b) alternative means to prevent GvH reactions are being explored.

PROGRESS: The program is continuing although Dr. Barrett, the L.R.F. fellow, has left. Early results indicate that BM transplants are well-tolerated by patients in first remission. Of 11 with AML in first remission there has been one relapse (an identical twin), two fatal GvH reactions, and eight continue in remission at periods of four weeks to 18 months; mild or chronic GvH is present in two.

187. FURTHER DEVELOPMENT OF CLINICAL BONE MARROW TRANSPLANTATION

Speck, B., Nissen, C., Gratwohl, A., Ruggero, D., Universitat Basel, Dept. of Internal Medicine, *Rheinsprung 9, Petersplatz 1, 4051 Basel, Switzerland*

OBJECTIVES: a) To determine if Cyclosporin A (CyA) can prevent GvH in allogeneic bone marrow transplantation for acute leukemia and aplastic anemia. b) To determine its efficacy in the treatment of chronic GvH.

APPROACH: a) All new recipients of allogeneic grafts are placed on prophylactic CyA. Its efficacy in preventing GvH and other complications is determined. Its influence on the hemopoietic precursor compartment and immune function is studied in vitro. b) Its therapeutic efficacy is studied in patients from previous series who are suffering from severe chronic GvH.

188. IMMUNOGENETIES AS APPLIED TO CLINICAL BONE MARROW TRANSPLANTATION

Blax, W. B., Johns Hopkins University, School of Medicine, Dept. of Medicine, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

This is part of a broader project. A summary of this subproject is not available.

189. SIGNIFICANCE OF NON-HLA ANTIGENS ON PLATELETS AND GRANULOCYTES IN BONEMARROW TRANSPLANTATION

Waters, A. H., Minchinton, R. M., Blood Transfusion Lab., *West Smithfield, c/o St. Bartholomews Hosp. MC, London EC1A 7BE, England, United Kingdom*

OBJECTIVE: The immunology of bone-marrow transplantation has focused on the lymphocyte and various techniques of pre-graft lymphocyte crossmatching and follow-up of graft rejection are highly developed. However, available evidence suggests that bone-marrow grafts represent a special case of transplantation immunogenetics and it is possible that non-HLA antigens on hemopoietic cells, such as platelets and granulocytes, may prove to be significant transplantation antigens.

Improved immunofluorescent antihuman globulin tests for demonstrating platelet and granulocyte antibodies have made it possible to examine the immunopathology of these cells in bone-marrow transplantation. It is proposed to use these new techniques to assess the significance of antibodies to platelet and granulocyte specific antigens in the pathogenesis of selective thrombocytopenia and granulocytopenia after bone-marrow transplantation.

APPROACH: Immunization against cell-specific antigens can be demonstrated by cross-matching the donor's cells with the recipient's serum using fluorescent antihuman globulin techniques. In some instances, especially in the case of autologous grafting, it may be relevant to react the patient's cells with his own serum in order to investigate the possibility of autoantibodies. Techniques include the platelet (PIFT), granulocyte (GIFT) and lymphocyte (LIFT) immunofluorescent tests, as well as the conventional lymphocytotoxicity test (LCT). The overall pattern of reactivity in these tests helps to define the specificity of the antibody.

The serological results will be correlated with hematology-peripheral cell counts and bone-marrow cytology.

190. TRANSPLANTATION OF BONE MARROW, FETAL LIVER OR THYMUS

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This is part of a broader project. A summary of this subproject is not available.

191. MARROW GRAFTING IN TREATMENT OF HEMATOLOGIC MALIGNANCIES

Storb, R. F., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

Over the past years the Division of Oncology at the University of Washington has been involved in a continuing program of marrow transplantation for the treatment of patients with advanced, otherwise fatal malignant and non-malignant hemopoietic diseases. A number of grafted patients have become long-term survivors without recurrence of their original disease and without occurrence of graft-versus-host disease. Many patients, however, have died from recurrent disease or, more frequently, from complications related to the transplant. Further improvement in clinical marrow grafting hinges upon progress in and better understanding of at least three major areas of study: 1) Recovery of immunologic reactivity in marrow graft recipients, 2) understanding of mechanisms of stable graft-host tolerance and of graft-versus-host disease, 3) methods of recognizing and overcoming sensitization of a marrow graft recipient by previous blood transfusions. It is the objective of this proposal to conduct an immunologic investigation into those three areas of study.

BIBLIOGRAPHIC REFERENCES: Atkinson, K., Meyers, J.D., Storb, R., Prentice, R.L. and Thomas, E.D.: Varicella-zoster virus infection after marrow transplantation for aplastic anemia or leukemia. Transplantation 29: 47-50, 1980. Ringden, O., Witherspoon, R.P., Storb, R., Ekelund, E. and Thomas, E.D.: Increased in vitro B-cell IgG secretion during acute graft-versus-host disease and infection. Observations in 50 human marrow transplant recipients. Blood 55: 179-186, 1980.

192. BONE MARROW TRANSPLANTATION IN HUMAN DISEASE

Santos, G. W., Johns Hopkins University, School of Medicine, Dept. of Medicine, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

The purpose of this program project grant is to investigate the immunobiology and improve the therapeutic effects of syngeneic, autologous, and allogeneic marrow transplantation in clinical malignancy and in disease involving bone marrow failure. The proposal is made up of five projects: (1) Therapeutic Application of Marrow Transplantation and Supportive Program Services; (2) Immunogenetics as Applied to Clinical Bone Marrow Transplantation; (3) Graft-Versus-Host Reaction and Disease (GVHD); (4) Autologous Lympho-hematopoietic Transplantation and (5) Radiotherapy as Applied to Lympho-hematopoietic Transplantation. The objectives of the various projects are: (1) Explore the therapeutic potential of cytoreductive treatment combined with syngeneic, autologous, and allogeneic marrow transplantation in human malignancy and aplastic anemia; (2) identify and characterize non-HLA and HLA-linked factors that are pertinent to engraftment and GVHD; (3) examine in depth the nature, prediction, prevention and treatment of GVHD; (4) further develop the methodology and study the effects of autologous lympho-hematopoietic transplantation in malignancy and (5) study the effects of fractionated radiotherapy together with chemotherapy in preclinical models.

193. A PROGRAM IN BONE MARROW TRANSPLANTATION

Gale, R. P., University of California, Los Angeles Campus, School of Medicine, Dept. of Microbiology & Immunology/ Medicine, *2025 Zonal Ave., Los Angeles, California,* 90024, United States of America

OBJECTIVE: Evaluate the role of bone marrow transplantation in the treatment of acute and chronic leukemia, solid tumors, aplastic anemia, and genetic disorders such as immunodeficiency diseases and thalassemia.

APPROACH: Several approaches are being evaluated in these diseases. These include: 1. Transplantation from HLA-identical siblings in patients with acute leukemia in remission. 2. Autologous marrow transplantation in patients with leukemia. 3. Phase 1-3 trials of marrow autotransplantation in patients with solid tumors. 4. Studies of alternative sources of hematpoietic stem cells for transplantation.

RESULTS: Results are summarized by treatment group: 1. 50-70 percent long-term disease-free survival in severe aplastic anemia. 2. 50 percent 2-year disease-free survival in acute leukemia in remission. 3. 50 percent response rates in patients with resistant solid tumors receiving marrow autotransplants.

194. RECONSTITUTION OF IMMUNOLOGIC REACTIVITY IN MARROW GRAFT RECIPIENTS

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This is part of a broader project. A summary of this subproject is not available.

195. ALLOGENEIC MARROW GRAFTING FOR TREATMENT OF ACUTE LEUKEMIA

Thomas, E. D., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington*, 98104, United States of America

This is part of a broader project. A summary of this subproject is not available.

196. BONE MARROW TRANSPLANTATION (HUMAN)

Graze, P. R., University of California, Los Angeles Campus, School of Medicine, Dept. of Microbiology & Immunology, *2025 Zonal Ave., Los Angeles, California*, 90024, United States of America

This is part of a broader project. A summary of this subproject is not available.

197. TOLERANCE OF BONE MARROW GRAFTS

Click, R. E., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Microbiology, *1305 Mayo, Minneapolis, Minnesota*, 55455, United States of America

The summary for this project is under entry no. 168.

198. A NEW APPROACH TO MARROW AND ORGAN TRANSPLANTATION

Slavin, S., Hebrew University of Jerusalem, Hadassah University Hospital, Dept. of Medicine, *P.O. Box 499, Jerusalem, Israel*

We are currently extending our observations on the immunoregulatory effects of total lymphoid irradiation (TLI), a procedure commonly used for the treatment of human malignant lymphomas. Incompatible bone marrow (BM) allografts can be accepted following pretreatment of recipient mice, rats and dogs with TLI (17 daily fractions of 200 rads), without evoking graft-versus-host disease (GVHD). During the past year, we have established permanent BM chimerism in parental yields F1 hybrids (C57BL/6 yields (C57BL/6 x BALB/c) F1) with 100% success, and to a lesser degree in complete allogeneic combinations (A/J yields (C57 x BALB) F1) using a modified short TLI protocol consisting of daily fractions of 200 rads each. Short TLI protocols will be further studied in large outbred animals to determine whether similar regimens could serve as a model for clinical BM and/or organ transplantation. In addition, we have characterized the tumor growth kinetics of the spontaneous, transplantable murine B cell leukemia (BCL1). We will use this animal tumor model to investigate adoptive immunotherapy of leukemia using TLI and allogeneic BM transplantation.

199. IRRADIATION AND MARROW TRANSPLANTATION IN LARGE ANIMALS

Thomas, E. D., Storb, R., Weiden, P., Deeg, J., Torok Storb, B., Sale, G., Shulman, H., Atkinson, K., University of Washington, School of Medicine, Dept. of Medicine, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington*, 98195, United States of America

This is a continuing study in the canine model of total body irradiation, marrow transplantation, methods of support for animals that have no bone marrow function and immunologic problems after marrow transplantation. The clinical marrow transplant protocols used in Seattle, and now being widely used in other medical centers, are to a considerable extent based on the results of past research supported by this grant.

The major areas of proposed research to be conducted under the renewed grant are as follows: (1) Prevention and treatment of graft-versus-host disease which is recognized as the principal obstacle to a wide application of marrow transplantation to human disease. (2) Studies of 'supralethal' fractionated total body irradiation designed to define the toxicity and immunosuppressive properties of fractionated irradiation with the objective of developing regimens that can be used to improve the cure rate of patients with acute leukemia. (3) Studies of the problem of resistance to hematopoietic grafts designed to overcome such resistance and improvement of the long-term success rate when donor-recipient pairs are not fully matched for the major histocompatibility complex. (4) Studies of lymphocyte-marrow interactions in vitro and in vivo in order to improve the success of marrow transplantation and also to provide information regarding graft-versus-host disease and stable graft-host tolerance.

200. GENETIC AND OTHER FACTORS AFFECTING MARROW TRANSPLANTATION IN IRRADIATED MICE

Uphoff, D. E., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Radiation Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Among the physical factors demontrated to affect the biological effect of x-irradiation are the qualitative differences between x-rays and the direction of the exposure, i.e., dorsal and ventral vs. two-directional. The dose rate was known to affect results when repair of radiation damage occurred during prolonged exposure. Dose rate was also responsible for the difference in acute double tube, acute dorsal exposure, and consecutive exposures from both dorsal and ventral sides which were administered within short exposures of as little as 12 minutes. The observed difference between dorsal and ventral exposures was attributed to a reduction in dose to vital hematopoietic tissue by absorption of x-rays as they pass through mouse tissue. Physical systems do not measure dose rate effects but may be used to demonstrate absorption of ionizing irradiation. In comparing the high energy irradiation from cesium-137 with that of x-rays it was found that a higher dose of gamma radiation was required to produce the same biological damage to hematopoietic tissue, however, there appeared to be less gut damage with increased exposure to Cs-137 than with the lower dose of x-irradiation.

201. BONE MARROW TRANSPLANTATION AFTER LY SUBSET ELIMINATION

Krown, S. E., Sloan Kettering Inst. for Cancer Research, Laboratory of Developmental Immunobiology, *410 E. 68th St., New York, New York*, 10021, United States of America

Using as a model the lethally irradiated mouse restored with allogeneic bone marrow from which particular Lyt and Lyb subsets have been serologically eliminated, with or without prior induction by lymphocyte precursors, we propose to determine the conditions under which immunologically competent chimeras free of GvH disease can be constituted. Our approach is based on the recognition that different, functionally-distinct subpopulations of lymphocytes can be distinguished and separated on the basis of their immunogenetic surface phenotypes. It also recognizes that agents such as thymopoietin and ubiquitin may induce committed lymphocyte precursors to express surface components characteristic of differentiated cells, and that these cells can then be eliminated by antiserum identifying lymphocyte subclasses.

Lethally irradiated CBA/J mice will be reconstituted with bone marrow from syngeneic (CBA/J), allogeneic H-2-incompatible (C57BL/6), or allogeneic H-2 compatible (congenic C57BL/6.H-2k) donors. (CBA X C57BL/6)F1 hybrids will also be used as bone marrow recipients to control for the contribution of HvG reactivity. Bone marrow will be transplanted either untreated, or after serological elimination of particular Lyt and Lyb subsets, with or without prior induction of lymphocyte precursors with thymopoietin or ubiquitin.

Mice so reconstituted will be compared for: 1) the severity of GvH reaction; 2) the restoration pattern of H-2-positive cells and of Lyt subpopulations in the spleen and lymph nodes; and 3) evidence of immunocompetence, measured by standard functional tests. Other studies will be performed to assess the effect of subpopulation elimination on the proliferative and differentiative capacity of hematopoietic stem cells and committed precursor cells, both in the donor marrow and in the reconstituted host.

202. BONE MARROW CYTOTOXIC PRECURSOR T CELLS

Klimpel, G. R., Rosse, C., Clagett, J. A., Henney, C. S., University of Texas, Medical Branch at Galveston, School of Medicine, Dept. of Microbiology, *300 University Blvd., Galveston, Texas,* 77550, United States of America

Bone marrow transplantation is viewed as a potentially invaluable tool for treating different cancers and immune deficiencies. However, little is known about the immunological functions of most bone marrow cells. This is evident from the fact that bone marrow transplantation is not always fully successful because of host-versus-graft responses (HVGR) and graft-versus-host disease (GVHD). The role that bone marrow pre-T cells play in these problems is unclear. In vitro models of GVHD and HVGR using bone marrow cells could greatly improve our understanding of these problems. I have developed an in vitro culture system which demonstrates that murine bone marrow cells can generate high levels of cytotoxic effector T cell activity alloantigens. This response may involve functional differentiation of bone marrow pre-T cells to cytotoxic effector T cells and/or helper T cells. The aim of this proposal is to investigate the cellular basis for this in vitro response and to determine whether or not bone marrow cells can generate in vitro cytotoxic responses against hapten-modified syngeneic cells or against parental hemopoietic histocompatibility (Hh) antigens. The reactive cells involved in these responses will be chracterized according to size, density, presence of Fc receptors, adherence properties, and for surface antigens associated with pre-T and splenic T cells. The expression of Thy 1 antigen and the development of cytotoxic T cell activity will be correlated for antigen stimulated Thy 1 negative bone marrow cells. Soluble factor(s) produced by activated T cells will be investigated for a possible role in this differentiation. Informaton obtained from these studies will not only add greatly to our understanding of bone marrow cells but will also provide information necessary for establishing in vitro models of GVHD and HVGR using bone marrow cells.

203. IMMUNOBIOLOGY OF BONE TUMORS AND BONE ALLOGRAFTS

Friedlaender, G. E., Yale University, School of Medicine, Dept. of Surgery, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

Osteosarcoma is the most common primary malignant bone tumor with a five-year survival of approximately fifteen percent following treatment by surgery and/or radiation therapy. Newer therapeutic approaches to this and other primary and metastatic bone tumors involve chemotherapy, immunotherapy, and bone allograft reconstruction of skeletal defects following less radical ablative surgery. Because of limitations imposed upon the size of the patient populations involved with each form of treatment by a large number of treatment protocols, and because of the ethical constraints of human experimentation, animal models have been developed by the principal investigator to study the immunobiology of osteosarcoma and bone allografts.

The objectives of this project are to evaluate currently proposed therapeutic protocols involving surgery, irradiation, chemotherapy, and immunotherapy in an animal model bearing clinical, histological, and immunobiological similarities to the human disease. The model is an initially localized virally induced (MSV) lesion produced in inbred neonatal rats (Wistar-Lewis). Using survival, histological onset of pulmonary metastatis, and an isotope release microcytotoxicity assay for humoral and cellular immunity as parameters of success, data will support the most efficacious treatment protocol, discourage less productive approaches, and perhaps suggest new directions for future therapeutics.

A rabbit model has also been established by the principal investigator in which the immunobiology of bone allografts required for post-ablation reconstruction can be evaluated. There is already data to suggest that the immunological response of the host is influenced by the methods of allograft preservation. This response can be measured by an isotope release microcytotoxicity assay and will be correlated with histological, radiological, and biomechanical information in order to clearly define the most acceptable method of bone allograft processing and long-term preservation.

Antigenicity of cartilage matrix and the effect of chemotherapy on bone biology and biomechanics are also being studied.

204. EXPERIMENTAL ALLOTRANSPLANTATION OF CARTILAGE AND BONE

Alho, A., Sondenaa, K., Bang, G., Nilsen, R., University of Bergen Hospital, Dept. of Surgery, *N5016 Bergen, Norway*

OBJECTIVE: To investigate possibilities of survival of preserved chondrocytes in hemijoint transplant which is used to fill a joint defect after tumor resection.

Condylar transplants of rabbit knee are preserved as follows: Equilibration in preservation medium containing glycerol and/or DMSO. Deep-freezing with various methods (minus 70 degrees to minus 196 degrees C). Auto- and allotransplantation of the preserved transplants.

D. OTHER STUDIES ON TRANSPLANTATION AND THE IMMUNE RESPONSE

205. THERAPEUTIC RESPONSE OF HUMAN TUMOR XENOGRAFTS

Steel, G. G., Peckham, M. J., Courtenay, V. D., Bateman, A. E., Phelps, T. A., University of London, *Senate House, London WC1E 7HU, England, United Kingdom*

Investigations will be performed mainly on xenografts of human tumors growing in immunosuppressed mice, and will be designed to elucidate the response of tumors to combined treatment with radiotherapy and chemotherapy. The usefulness of xenografts will be evaluated, concentrating upon carcinoma of the pancreas and testicular tumors. Comparisons will be made with Lewis lung carcinoma and with the response seen in patients. Emphasis will be placed on the search for in vivo evidence of potentiation between cytotoxic drugs and radiation. The results will be correlated with tumor size, both at the start of treatment and during regression. Parallel studies will be made of normal tissue reactions in lung, bone marrow and intestine, and in a subsidiary investigation the action of electroaffinic radiosensitisers will be tested on the xenografts.

206. HETEROTRANSPLANTATION OF HUMAN HEAD AND NECK CANCER IN THE NUDE ATHYMIC MOUSE - A POTENTIAL MODEL FOR ANTI-CANCER DRUGS

Sneeuwloper, G., Snow, G. B., Veldhuizen, R., Universiteit van Amsterdam, Dept. of Experimental Medicine/Otolaryngology, *Spui 21, Amsterdam, Netherlands*

OBJECTIVE: Evaluation of the effects of cytotoxic drugs commonly used in treating head and neck cancer patients and screening of 'new' drugs in head and neck squamous cell carcinoma transplanted in athymic nude mice.

APPROACH: Transplantation of human head and neck cancer in nude athymic mice: When tumors in the mice reach a size of 100-150 mm3 cytotoxic drugs are administered according to various dose schedules. Tumor volume is used as a parameter of the antitumor effects; serial transplantations are carried out.

207. ENHANCEMENT OF ORAL CANCER AFTER ALLOGRAFTING

Pliskin, M. E., University of Pennsylvania, School of Dental Medicine, Dept. of Pathology, *4001 Spruce St., Philadelphia, Pennsylvania,* 19104, United States of America

The proposed investigation concerns itself with the observation that organ allograft recipients have a greater risk of developing squamous cell carcinoma of the labial mucosa as well as other types of cancer.

I hypothecate that the increased incidence of cancer is, at least in part, a result of the generation of suppressor cells in-

duced by the allograft antigens and that these cells nonspecifically reduce the immune response to nascent tumors arising in the allograft recipients. This hypothesis is supported by preliminary experimental evidence presented by myself and others.

The need for this hypothesis has been necessitated by the numerous investigations which have shed doubt on the efficacy of the classical immunological surveillance system as an explanation for the observed increased incidence of neoplasia in allograft recipients. In order to support my hypothesis, four major questions are asked in this proposal: 1. Do allografted mice have an enhancement of syngeneic tumor growth? 2. Is the anti-tumor immune response reduced in the allografted recipients? 3. Is the reduction of the anti-tumor immune response due to suppressor cells within the spleens of the allografted mice? 4. Are the suppressor cells T cells or macrophages?

208. IMMUNOLOGICAL ENHANCEMENT OF RENAL ALLOGRAFTS

Stuart, F. P., Fitch, F. W., University of Chicago, Pritzker School of Medicine, Dept. of Surgery, *5801 S. Ellis Ave., Chicago, Illinois*, 60637, United States of America

The overall objective of the total project is to elucidate mechanisms involved in induction and maintenance of allografts in the rat. We have reported that survival of Lewis/BN allografts in Lewis recipients can be enhanced by treating the recipient with Lewis anti-BN alloimmune serum or Lewis/BN spleen cells or both. A spectrum of enhancement can be achieved by varying the dose, timing, and quantity of these biological reagents. However, the optimal regimen appears to be intravenous administration of 5 x 10 to the 7th power LBN (donor strain) spleen cells 11 days before transplantation and one ml. of hyperimmune Lewis anti-BN serum 10 days before transplantation. This regimen stimulates peak titers of an anti-idiotypic response against anti-BN receptors in the recipient by the day of transplantation. During the past two years most of our effort has been to generate mouse-rat monoclonal hybridoma antibodies of Lewis anti-BN specificity in an attempt to define more clearly the requirements of allograft enhancement. We have generated and tested more than 20 monoclonal mouse-Lewis hybridoma anti-BN supernatants. All are directed against Class I histocompatibility antigens. Four of them led to allograft enhancement when combined with donor strain LBN spleen cells in the -11 day, -10 day regimen. None of the supernatants enhanced graft survival when given alone. We propose to continue attempts to generate a mouse-Lewis rat hybridoma with specificity against Class I BN histocompatibility antigens. We will also combine various effective supernatants in an attempt to achieve enhancement without the need for concomitant donor strain spleen cells.

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209. MATERNAL TRANSMISSION, ROLE IN KIDNEY TRANSPLANT AND HEPATOMA

Knudson, A. G., Inst. for Cancer Research, *7701 Burholme Ave., Philadelphia, Pennsylvania*, 19111, United States of America

This is part of a broader project. A summary of this subproject is not available.

210. NATURAL RESISTANCE BY NON-IMMUNIZED MICE AGAINST LEUKEMIA TRANSPLANTATION - GENETICS, MECHANISMS, AND IMPLICATIONS

Carlson, G. A., University of Alberta, Dept. of Immunology, *Edmonton, Alberta*, T6G 2E1, Canada

OBJECTIVES: a) To further characterize natural resistance by non-immunized mice against transplantable leukemias. b) To determine whether paraimmune defense mechanisms operate as a primitive surveillance mechanism against neoplasia. c) To study the role of natural resistance in the subsequent development of immune responses against tumor-specific and alloantigens.

APPROACH: By monitoring the fate of leukemia cells which have been prelabelled with 131I-iododeoxyuridine, natural resistance against semisyngeneic and allogeneic cells can be studied in vivo. Mice which are not H-2 identical with the leukemia cells rapidly recognize and kill transplanted cells as indicated by decreased leukemia cell survival (determined by whole-body gamma counting) and by inhibition of splenic colonization by the leukemia. In some strains of mice, resistance is abolished by whole-body irradiation while in others, resistance is left intact. Genetic and mechanistic analyses will be used to further characterize natural resistance as it involves both the target cell and the host, with emphasis on the specificity of the reaction.

Determination of whether natural resistance could operate as a primitive surveillance mechanism will involve studies on resistance to syngeneic leukemias, including resistance by congenitally athymic mice.

The effects of natural resistance on the subsequent development of immune responses will be studied by determining the effects of abrogation of resistance on the classes of immunity induced and by comparing the response to tumor-specific antigens in radiosusceptible and radioresistant F1 hybrid mice against parental strain tumors.

PROGRESS: Recent experiments have indicated that natural resistance in both normal and irradiated mice involves macrophages, is T-independent, matures later than the ability to mount an alloimmune response, and is strontium-89 sensitive.

211. HEREDITARILY ATHYMIC-ASPLENIC MICE - TUMOR GROWTH

Lozzio, B. B., Machado, E. A., Lozzio, C. B., Herberman, R. B., University of Tennessee, Center for the Health Sciences, Memorial Research Center & Hospital, *1924 Alcoa Hwy., Knoxville, Tennessee*, 37920, United States of America

This project is a continuation of our current studies on the heterotransplantation of human malignancies in immunodeficient animals, namely athymic (nude) and asplenic-athymic (lasat) mice. Chronic myelogenous leukemia (CML) cells with the Philadelphia chromosome from the cell line K-562 proliferate forming solid tumors (myelosarcomas) in nude mice associated with extensive metastatic proliferation when transplanted in lasat mice. The treatment of mice with xenoantisera to K-562 cells suppressed the growth of myelosarcomas. On the basis of these findings we propose to use the human cancer cell-animal tumor systems described above as models for the formation and subsequent growth of solid tumors, with the emphasis on the conditions of the host and/or the malignant cells required for local growth and metastatic proliferation.

The specific aims are: 1) To continue our current studies on the heterotransplantation of K-562 cells, 2) to continue to improve the treatment of myelosarcomas and metastases made of K-562 leukemia cells with specific antibody to K-562 cells, 3) to determine the mechanisms of action of the successful passive immunotherapy for myelosarcomas and metastatic proliferations of K-562 cells, 4) to develop drug-antibody conjugates for the treatment of mice bearing myelosarcomas and metastases, 5) to determine whether or not the natural cytotoxicity of nude mice is a major tumoricidal mechanism preventing the local growth and metastatic proliferation of myelosarcomas and other solid tumors of human origin mentioned in (6), 6) to develop new procedures for the heterotransplantation of human lung, breast and colorectal carcinomas, melanomas and the terminal blastic crisis of a CML (these malignancies are the primary targets of concern because there is no reliable treatment; they are the most common types of cancer, very difficult to transplant in nude mice), and 7) to treat mice bearing human tumors with current and/or new therapeutic agents other than xenoantisera and antibody-drug conjugates. We propose various innovative approaches and novel ideas for the heterotransplantation of human malignancies.

212. INDUCTION-CONTINUATION-GENETICS OF EXPERIMENTAL TUMOR

Morris, H. P., Howard University, School of Medicine, Dept. of Biochemistry, *2400 6th St. N.W., Washington, District of Columbia*, 20001, United States of America

It is proposed that a central depository of pedigreed transplantable hepatoma tumor lines be maintained and disseminated from Howard University Medical School where complete records are available for any information needed by basic cancer

research investigators. The spectrum of transplantable tumors is made up of more than 30 different tumor lines. The hepatomas differ in growth rate by more than 20-fold. They are divided among slow growing, highly differentiated; intermediate growth rate, well-differentiated; and fast growing, poorly differentiated tumor lines. Investigators from all parts of the United States and abroad make requests for suitable basic cancer research materials and are advised as to which hepatoma lines are most suitable for the type of research they propose. In addition, investigators at Howard University Medical School make use of this spectrum of tumors. We propose to induce adenocarcinomas in the regenerating liver of the rat after a single dose of strongly acting chemical carcinogens; chromosome banding analyses will be made of a selected spectrum of tumors; the hybridization of rat hepatoma cells and mouse cells will be performed in attempts to relate specific chromosomes or a specific complement of chromosomes to malignancy; an evaluation of hepatoma microtubule structure and mitotic spindle formation will be accomplished; development of frozen storage-retrieval techniques will be carried out on this spectrum of transplantable tumors; studies on the mechanism of metastasis formation will be performed.

213. TRANSPLANTATION ANTIGENICITY OF VIRUS INDUCED TUMORS

Hellstrom, I. E., Nishiyama, K., Woodbury, R. G., Fred Hutchinson Cancer Research Center, Division of Tumor Immunology, *1124 Columbia St., Seattle, Washington*, 98104, United States of America

We are continuing our work on humoral and cell-mediated immune reactions to human tumor-associated antigens. A major effort at this time goes into using the monoclonal antibody technique to detect and characterize cell surface antigens of various human tumors, particularly melanomas. So far, antigens of very restricted distribution, as well as antigens shared by many tumors, have been detected. A particularly interesting antigen, p97, has been demonstrated in about 90% of human melanomas and about 50% of other human tumors, but has not, so far, been detected in normal human tissues. We are attempting to develop radioimmunoassays for this antigen, to study its distribution further, to investigate to what extent it is expressed in vivo, whether it can undergo antigenic modulation, and whether a p97 positive tumor gives rise to variants which lack p97. Similar studies are being conducted with p210, another human tumor-associated antigen, which we recently identified; p210, has, so far, been less studied for specificity. Several other hybridomas forming antibodies reacting with cell surface components of human tumor cells, are presently being worked up. The relationship, if any, between antigens detected by the hybridoma technique, such as p97, and antigens recognized by the patients (in the form of targets for humoral or cell-mediated reactivity) will be investigated, using various blocking assays.

Other studies concern the targets of cell-mediated immune responses of Moloney virus-induced sarcomas, as outlined in my original grant application.

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214. IMMUNE RESPONSE TO MURINE TRANSPLANTATION AND TUMOR ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

215. IMMUNOLOGIC CHARACTERIZATION/PROSTATIC ADENOCARCINOMA

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Classical transplantation-resistance experiments designed to determine whether the R3327 tumor induces specific immunity in syngeneic hosts were performed, but because of technical difficulties have not yet yielded convincing evidence to suggest that the R3327 tumor is immunogeneic. Further experiments are currently under way to obtain more data.

Experiments to test whether non-tumor-antigen-specific immunologic mechanisms might also play a role in transplantation resistance to the R3327 tumor have shown that carrageenan treatment of animals before and after tumor inoculation reduced the capacity of nonimmune host to reject the R3327 tumor. In vitro studies on the mechanism of action of carrageenan suggest that carrageenan treatment reduces natural killer cell activity in splenic lymphocytes; however, these experiments have not yet been performed using R3327 target cells in the assays. Other experiments indicate that carrageenan treatment does not alter serum hemolytic complement titer in the protocol used.

We have now established cell lines of both the anaplastic (more than 80 passages) and the hormone-dependent (10 passages) varieties of the R3327 tumor, and plan to use these cell lines as target cells in our future in vitro cytotoxicity assays.

216. IMMUNOLOGICAL EFFECTS ON TUMOR GROWTH AND REJECTION

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Evidence for MHC restriction of soluble helper effects was observed in the generation of syngeneic killer T cells to TNP-altered self. Ia bearing T cells obscure the observation of such interactions and, thus, must be removed in order to detect MHC restriction of 'nonspecific' soluble helper factor supernatants. Genetic mapping studies demonstrated that the strain producing HFS must be compatible in the H-2IA, H-2IB region of the H-2 MHC with the strain utilizing the helper molecules in order for optimal helper signals to be delivered.

217. ANALYSIS OF MECHANISMS OF HUMORAL AND CELL-MEDIATED IMMUNITY TO ALLOGRAFTS AND TUMORS

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OBJECTIVE: Study of the mechanism of in vitro induction and of the in vitro and in vivo functional activity of cytolytic T lymphocytes (CTL) in allograft and MSV induced tumor immunity in the mouse.

APPROACH: Generation of CTL in mixed leukocyte and in mixed leukocyte-tumor cell culture. Use of cell surface markers, antigen binding and cell separation techniques, and of functional assays in the characterization of responding and accessory cell types. Study of the role of nonspecific factors in CTL generation. Study of clonally expanded CTL populations and of T-cell hybridomas. Study of the specificity of individual responding T cells in limiting dilution assay.

PROGRESS: Highly cytolytic T lymphocytes were generated in vitro by repeated stimulation with allogeneic lymphocytes or with MSV induced lymphoma cells. Clonal expansion of CTL was achieved by further stimulation with nonspecific factors generated in secondary MLC. A limiting dilution assay was developed, allowing the determination of frequency and specificity of individual alloreactive and MSV tumor reactive CTL precursors.

218. MECHANISM OF DELAYED HYPERSENSITIVITY AND TUMOR GRAFT REJECTION

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The summary for this project is under entry no. 162.

219. IMMUNITY TO A TRANSPLANTABLE LYMPHOID TUMOR OF CHICKENS

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OBJECTIVE: Determine the relative importance of humoral and cellular immunity in the detection of a transplantable lymphoid tumor (TLT) of chickens. Develop a reliable assay of cellular immunity to TLT. Determine the cell types responsible for the rejection of TLT.

APPROACH: A flock of at least 20 hyperimmune chickens will be established and will serve as the donors of immune serum and cells. TLT will be maintained by weekly passage in susceptible 3-4 week old chicks. Preliminary investigations of suitable cytotoxicity assay have indicated that a by-stander population of chicken erythrocytes may be used as an indicator of cell-mediated immunity against TLT. Another possibility is the use of a radioactive label of TLT cells. TLT has an affinity for iron and the tumor cells can be labeled in vivo with Fe. However, the suitability of this label in vitro has yet to be determined. Once a useful cytotoxicity assay system has been developed, it will be used to determine the role of interactions of passively administered immune serum with cells of the host's immune system, as well as the cell types involved. In addition, selective manipulation of the chicken's immune system by chemicals and radiation will be used to study the relative importance of populations of immune cells in the rejection of TLT.

PROGRESS: A trial was conducted to evaluate the effect of niridazole on mortality due to the transplantable lymphoid tumor (TLT). Niridazole at oral doses of 50 mg/kg body weight suppressed cellular immunity in chickens. Each of 40 six week old chickens received niridazole on 4 consecutive days. At 7 weeks, 150,000 TLT cells were injected into the pectoral muscle of 20 control and 20 niridazole-treated chickens. In the 5-week trial period, 60% of the niridazole-treated TLT-bearing chickens died with a mean survival time of 13.4 plus or minus 3.9 days. Ninety percent of the control TLT-bearing chickens died with a mean survival time of 17.1 plus or minus 5.9 days. At the time of TLT inoculation, responses of lymphocytes of niridazole-treated chickens to phytohemagglutinin (PHA) were reduced by 60% compared to controls. The active metabolite of niridazole is present in plasma. (Text Abridged.)

220. IMMUNE RESPONSE TO TUMOR GRAFTS

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Our principle goal is to determine the physiological mechanisms which regulate suppressor T cell activity. To do this, we separate suppressor T cells from other T cells with the use of monoclonal antibodies against differentiation antigens. We then separate other subsets of cells and mix them with the suppressor T cells. After incubation of the two populations of cells we remove one or the other with the appropriate antiserum against unique differentiation antigens and ask (1) how the suppressor T cells have affected the function of the other cell set with which they were mixed and (2) the reciprocal. Besides mixing cells and assaying T cell activity, we are attempting to isolate and characterize the biologically active mediators that these T cells use in communicating with one another.

VI. BROAD PROGRAMS FOR HISTOCOMPATIBILITY STUDIES AND TRANSPLANTATION BIOLOGY

221. STUDIES ON TRANSPLANTATION ANTIGENS AND OTHER CELL SURFACE ANTIGENS

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OBJECTIVE: The classical histocompatibility antigens (HLA-A, B, C, H-2K, D, etc.) are highly polymorphic cell surface components which constitute the target molecules recognized by T-killer lymphocytes in graft rejections. We have earlier shown that these classical transplantation antigens share structural features with immunoglobulins. One of the main objectives of the present study is to investigate if the transplantation antigens are immunoglobulin-like also functionally.

APPROACH: We have clearly shown that a virus-coded protein in adenovirus-infected cell lines is associated with transplantation antigens on the cell surface. We intend to continue these studies in order to elucidate the nature of that molecular interaction. Moreover, we want to investigate if the transplantation antigen virus protein is recognized by T-killer cells in order to obtain a molecular explanation of the immunological rejection of tumor cells. We also plan to investigate the primary structure of the transplantation antigens in order to clarify if the genetic polymorphism is located in clusters in the molecule or scattered throughout the whole structure. We would like to correlate these findings with the existence of hyper-variable regions in immunoglobulins.

Studies of transplantation antigen-like molecules which are expressed early during the autogenesis will be continued. We plan to isolate these molecules in chemical amounts in order to try to elucidate in detail their structural similarities to the classical transplantation antigens.

222. CELL SURFACE ANTIGENS OF MURINE TUMORS

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The aim of this proposal is to investigate the biological and chemical profile of murine major histocompatibility (MHC) antigens (H-2 and Ia) on neoplastic cells. Since it has been demonstrated that there are alterations of H-2 and Ia antigens on tumor cells, a concerted effort will be made to characterize the expression of these MHC antigens. In addition the biochemical makeup of both MHC antigens and tumor associated antigens (TAA), as well as their relationship to one another, on tumor cells will be determined and compared with that of MHC antigens on normal cells. Attempts will be made to assess the capacity of these antigens to elicit cellular and humoral immune responses in order to evaluate the effect of malignant transformation on these cell surface markers. Xenoantibodies to tumor associated antigens will be used to develop sensitive radioimmunoassays for TAA and to compare the cross-reactivity of TAA from various tumors. Circulating TAA will also be biochemically and immunologically characterized in order to evaluate their role in the pathology of malignant disease. In addition, the appearance and quantity relationship to circulating MHC antigens will be determined and correlated with stages and locations of tumor growth. A study of quantitative changes in the expression of MHC antigens on tumor cells, as well as their relationship with neoantigens of the tumor, will provide information essential to an assessment of the role of these antigens in cell communication and control phenomena. In addition a precise determination of the time course of appearance of altered and neo-antigens both on the cell surface and in the serum will allow for a critical evaluation of the potential of these changes for immunodiagnosis and immunotherapy.

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223. CELL-SURFACE ANTIGENS OF MURINE TUMORS

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Malignant transformation is accompanied by extensive cell surface alterations. Of particular interest are alterations of histocompatibility antigens, cell surface components which have been implicated in cell-cell interactions in the immune response and in histogenesis. This project will employ serological and immunochemical techniques to characterize cell surface antigens of a series of spontaneous and chemically induced murine tumors. The antigenic profiles of the tumor cells will be analyzed by 2-dimensional electrophoresis of immunoprecipitates. Specific antisera will be used to assess the contribution of fetal and viral antigens to the total antigenic mosaic. Histocompatibility antigens expressed by tumor cells will be subjected to extensive immunochemical analysis in order to detect structural alterations. Immunofluorescence techniques and bivalent crosslinking

reagents will be used to examine topographic relationhips between histocompatibility and tumor-associated antigens on the cell surface. Serum from animals bearing tumors will be examined for the presence of tumor associated and histocompatibility antigens. These studies will contribute to our understanding of the structural basis of altered-self recognition in relation to tumor immunity and should provide insight into the role of cell surface structures in regulation of cell proliferation and positional control.

224. IMMUNOBIOLOGY OF THE LABORATORY RABBIT

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This program for the continued development of the laboratoy rabbit as a research tool emphasizes inbreeding, the immunogenetic characterization of inbred or selectively bred animals, and the use of these animals for studies of the histocompatibility systems, transplantation of organs and as a model for tumor immunology. We continue to make available characterized animals, tissue and red cell typing service and typing reagents to all qualified investigators. Studies on the major histocompatibility system of the rabbit have now progressed to the study of the cross reacting groups of antigens which are detected by serological methods and their relationship to the specificities detected by the mixed lymphocyte culture test. It appears that the serological test and MLC reaction are detecting the products of a single locus or of a pair of very closely linked genes. It is through the partitioning of the complex array of antibodies obtained by cross immunization by tissues that we hope to gain a better understanding of this animal model of the D-D loci of the human HLA system. A large variety of isoimmunization are underway and the resulting sera are being tested against subsets of lymphocytes and other formed elements of the blood in an effort to elucidate the fine structure of the rabbit major histocompatibility complex. Related to the studies of histocompatibility is our effort to describe marker genes located in the area of MHC through our study of serological or biochemical polymorphisms in the rabbit.

BIBLIOGRAPHIC REFERENCES: Hagen, K.L., Young, L.E., Tissot, R.G. and Cohen, C. 1978. Factors Affecting Natural Antiallotype Antibody in Rabbits. Immunogenetics 6:355-366.

225. TRANSPLANTATION ANTIGENS OF SWINE

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A breeding program has been carried out starting with two miniature pigs from different sources and selecting offspring according to tissue typing procedures aimed at defining the major histocompatibility complex of this species. By this procedure three herds of miniature swine, each homozygous for a different set of histocompatibility antigens at the MHC have been developed. Current projects include: 1) Assessment of survival of organs and tissue transplants among and between members of these herds as a model for tissue typing and transplantation; 2) purification and characterization of the major histocompatibility antigens of this species, and isolation and characterization of peptides from these antigens for sequence analyses and for assessment of immunologic reactivity; 3) assessment of the immunologic parameters involved in tolerance to liver allografts in this species; 4) detection of intra-MHC recombinants; and 5) immunizations with purified immunoglobulin preparations between members of different herds in an attempt to produce antiallotype reagents. Two intra-MHC recombinants have been obtained and are being bred to homozygosity. These should permit assessment of the effects of matching at different MHC regions on the outcome of transplantation in this animal model.

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226. HISTOCOMPATIBILITY MUTATIONS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

227. TRANSPLANTATION BIOLOGY (HUMAN, DOGS, RATS)

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This is part of a broader project. A summary of this subproject is not available.

228. TRANSPLANTATION IMMUNOLOGY

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The long-term objectives of the studies proposed in this application are to define the roles of various classes of antibodies in the induction of immunologically mediated tissue damage and to devise procedures for the selective stimulation of individual classes of antibodies in response to cell surface antigens. This information will be obtained through the use of transplanted tissues as targets for potentially destructive or protective antibodies. We plan also to study the mechanisms involved in antibody-mediated destruction or protection (enhancement) and to investigate the genetic determinants of responsiveness insofar as they involve differences in the kinds of antibodies formed in response to cell surface antigens.

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229. TECHNIQUES FOR LIVER TRANSPLANTATION (DOGS)

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This is part of a broader project. A summary of this subproject is not available.

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